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Surveillance for *Dirofilaria* spp. in American Black Bears (*Ursus americanus*) in Pennsylvania, 2018-2020.

ALIA RENEE MAZZATOSTA
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Reviewed and approved* by the following:

Justin Brown
Assistant Teaching Professor and Program Coordinator of Veterinary and Biomedical Sciences
Thesis Supervisor

Robert J. Van Saun
Extension Veterinarian and Professor of Veterinary Science
Honors Adviser

* Electronic approvals are on file.

ABSTRACT

Dirofilaria ursi is a filarial nematode of bears, including American black bears (*Ursus americanus*), grizzly bears (*Ursus arctos*), and Asiatic black bears (*Ursus thibetanus*), that is vectored by black flies (*Simuliidae*). Clinical disease associated with *D. ursi* infection has not been reported in bears. Infection with *D. ursi* in bears can be detected by examining the subcutis and body cavity for adult nematodes or the blood for microfilaria. Surveillance based on detection of adults can underestimate infection in a population because of the highly variable anatomic location of the worms. Consequently, surveillance for *D. ursi* in this study was performed by screening blood for microfilaria. Blood smears from 129 black bears from Pennsylvania were examined, and 33 (25.6%) were found to contain microfilaria morphologically consistent with *Dirofilaria* spp. Other than bears that had sarcoptic mange, none of the positive bears had any reported overt signs of disease or lesions. Age, sex, and season did not have a significant effect on black bears being positive for *Dirofilaria* spp. ($p > .05$). Black bears that had sarcoptic mange were significantly less likely to be positive for microfilaria than bears without mange ($p < 0.05$). The results of this study indicate *Dirofilaria* spp. infection is common in black bears in Pennsylvania, but is not associated with disease. This data is consistent with previous surveys conducted in black bears in the Upper Midwest. Future studies are needed to further characterize epidemiologic patterns of *Dirofilaria* spp. infection in black bears, define associations between other microorganisms (e.g. *Wolbachia* and *Sarcoptes scabiei*), and determine the zoonotic potential of this common nematode of bears.

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LITERATURE REVIEW

Introduction

As a result of habitat loss (forest conversion to agricultural land) and overharvest, American black bear populations in Pennsylvania reached critically low levels and were geographically restricted after English settlement in the 1800s. Conservation efforts, habitat restoration, and hunting regulations implemented over the last century have resulted in the recovery of black bears in Pennsylvania, which now are found throughout most of the Commonwealth and have a statewide estimate of 18,000 bears (Ternent, 2019).

For wildlife populations, carrying capacity refers to the size of the population that can be supported without adverse impact. One component of carrying capacity is biological carrying capacity, which is the number of animals that a habitat can sustain with the given resources (e.g. food, water, cover, etc.) without negative impact on the host or environment (Seidl & Tisdell, 1999). Measures of a wildlife population exceeding the biologic carrying capacity include poor nutritional condition, reproductive declines, habitat destruction, and/or increasing disease. There is currently no evidence to suggest that black bears are reaching the biologic carrying capacity in Pennsylvania.

The other important component of carrying capacity is wildlife acceptance capacity, which is the population of wildlife that people will accept in an area (Decker & Purdy, 1988). As a result of the increasing and expanding population, black bears in Pennsylvania are more likely to interact with humans, domestic animals, and/or other wildlife. Many of these interactions are

positive (e.g. increased hunting and wildlife viewing opportunities); however, some are negative (e.g. property destruction or aggressive physical interactions with domestic animals). Bear-to-human and bear-to-domestic animal interfaces will presumably continue to increase as the population grows, as will the potential for negative interactions. These negative interactions, and the associated public opinions, represent a significant factor in determining the wildlife acceptance capacity of black bears in Pennsylvania. Consequently, wildlife acceptance capacity is an important consideration for managing the increasing black bear population in Pennsylvania.

Diseases have historically been an uncommon source of mortality in black bears relative to human-associated fatalities, such as vehicle collisions, hunting, or nuisance removals. However, risk factors associated with infectious and non-infectious diseases can change as a result of increasing black bear populations and/or new interfaces between bears, humans, animals, and wildlife. Black bears are hosts for a wide diversity of pathogens, including viruses, parasites, and bacteria. In most cases, infections with these pathogens do not cause overt disease or negative impacts to bears. However, some of these pathogens may be harbored by bears asymptomatically, but produce disease when they spillover into humans, domestic animals, or other wildlife. Consequently, it is important to define and monitor the presence of pathogens and occurrence of disease in black bears in Pennsylvania.

Dirofilaria ursi is a filarial nematode found in American black bears, grizzly bears, and Asiatic black bears. Currently, there is no evidence that *D. ursi* causes disease in black bears; however, there are sporadic reports of skin nodules in humans associated with intralesional *D. ursi*-like nematodes. *Dirofilaria ursi* has been reported in black bears in the northern United States, but the prevalence and epidemiologic trends are undefined. The objective of this literature review is to provide a summary of information on *Dirofilaria* spp.in black bears.

American Black Bears in Pennsylvania

The recovery of American black bears (*Ursus americanus*) in Pennsylvania over the last 50 years is a testament to sound wildlife management. Since the early 1980s, the population of black bears in Pennsylvania has increased from 4,000 to over 18,000 bears (Pennsylvania Game Commission, 2021), and their geographic range has expanded from populations largely restricted to the north central and northeastern parts of the state to about 54 counties throughout Pennsylvania (Ternent, 2006; Ternent, 2019).

Biology

American black bears are typically black, but other color phases exist including cinnamon, blonde, and brown. Bears can adapt to a diversity of habitats, but generally utilize wooded landscapes, often favoring heterogeneous areas that include both forest cover and openings (Ternent, 2006). They are opportunistic omnivores that will feed on a diversity of items depending on season and availability. Up to 75% of their diet is typically vegetation, with the rest consisting of animal matter and invertebrates (Ternent, 2006). Black bears exhibit solitary behavior driven by dominance hierarchy (Rogers, 1977). They are polygamous, with the male and female only coming together during breeding season to mate. In Pennsylvania, breeding season occurs during the summer (June-July) and sows will give birth to an average of 3 cubs (range 1-5) during early January while the sow is hibernating. Sows generally have cubs every other year. Sows also exhibit a reproductive phenomenon called delayed implantation or embryonic diapause (Wimsatt, 1963), in which the embryo remains in a state of dormancy and delays implantation into the uterine wall after fertilization. This reproductive strategy allows

pregnancy to be delayed until after the fall foraging period to ensure that the cub births will only proceed if adequate resources are available and appropriate nutritional status of the sow is achieved (Ternent, 2006). After the cubs are born, they nurse and remain with the sow in the den until the group emerges in early Spring (March-April). Cubs remain with the sow throughout that following year and go back into the den with the sow as yearlings (1 year old). Once they emerge from the yearling dens, the group will disperse, and the sow will breed again that summer (Ternent, 2006).

History

Black bears were once a common and prevalent species throughout much of Pennsylvania. However, black bear populations in the Commonwealth experienced a steady decline around the time of European settlement in the 1800s. Bears were harvested by early settlers and utilized as an important source of food, fur/hide, and fat; however, like many North American game species, bears were over-harvested (Ternent, 2006). To compound this excessive hunting pressure, woodland habitats throughout Pennsylvania were converted to farmland and trees harvested for timber. This combination led to significant declines in the black bear populations that reached between 2,000 and 4,000 bears in the first half of the 1900s. As a result of this population decline, bear hunting in Pennsylvania was completely halted in 1970, 1977, and 1978 due to the critically low bear population estimates. Wildlife management initiated in 1905 began to address these negative trends in black bear populations in Pennsylvania, including the establishment of bear hunting regulations (1911), creation of a distinct bear hunting tag (mid 1970s), and intrastate reintroduction efforts (1923-1926 and 1979-1984). These efforts were

complemented by habitat management that was occurring throughout the Commonwealth to regenerate forests and reestablish woodland habitats. Collectively, these efforts have led to a steady increase in black bears throughout Pennsylvania since the early 1980s. The statewide bear population is now estimated to exceed 18,000 (Pennsylvania Game Commission, 2021; Ternent, 2019), and bears have been found in every county in Pennsylvania, with higher concentration in north-central and northeastern areas (Ternent, 2006).

While the population increase and geographic expansion of black bears in Pennsylvania is a wildlife success story, it has also resulted in some negative conflicts and questions relating to how many bears can be sustained in the Commonwealth. Carrying capacity is a term that refers to the maximum number of individuals in a population that can be supported by the surrounding area without negative impact (Ellen, 1982). Biological carrying capacity is the maximum population that can be supported by the environment without negative implications on the population and environment (Seidl & Tisdell, 1999). Measures of exceeding the biological carrying capacity include poor nutritional condition, reproductive declines, habitat destruction, or disease (Liu & Borthwick). To date there is no evidence that black bears in Pennsylvania are exceeding the biological carrying capacity. Wildlife acceptance capacity is the maximum population that can be reached without annoyance or harm to the general public living in the area (Decker & Purdy, 1988; Seidl & Tisdell, 1999). Measures of black bears approaching or exceeding wildlife acceptance capacity in Pennsylvania include negative bear-to-animal interactions, including physical confrontations and risks for disease transmission, or negative human-to-bear interactions, including vehicle collisions, physical confrontations, and threatening behaviors by bears. A telephone survey conducted in 2008 of Pennsylvania residents 18 years of age or older by Responsive Management (Responsive Management National Office in

Harrisonburg, Virginia) for the Pennsylvania Game Commission indicated that reaching the wildlife acceptance capacity is a potential concern for black bear management in the Commonwealth (Responsive Management, 2008). In the survey, 59% of participants said that they want the bear population to remain the same, while 12% hope for an increase and 12% hope for a decrease. Additionally, people were concerned with direct contact with bears; only 15% are comfortable having black bears in their yard. Reaching the wildlife acceptance capacity likely represents the most likely limiting factor in the future for the thriving black bear population in Pennsylvania.

Black bear populations are actively managed in Pennsylvania to maintain bears at sustainable and huntable levels, while minimizing human-bear interactions or other measures of exceeding carrying capacities (Ternent, 2006). The Pennsylvania Game Commission has a Black Bear Management Plan that outlines goals, objectives, and strategies for bear management in the Commonwealth, and this plan is updated every 10 years (Ternent, 2006). The primary means for managing the size of the black bear populations is through regulated hunting. However, additional complementary measures are employed to maintain positive public opinions on bears and prevent negative human-bear interactions, including banning the practice of feeding bears and providing education, outreach programs, and documents on how to appropriately live around bears (Pennsylvania Game Commission, 2021). In addition, measures of reaching the biological carrying capacity (disease, nutritional condition, reproduction) or wildlife acceptance capacity (human- or domestic animal-bear conflicts) are monitored each year in order to track trends that may warrant management changes (e.g. extended hunting seasons or increased public education efforts).

Mortality of Bears in Pennsylvania

The most common causes of mortality in black bears in Pennsylvania are associated with humans. Hunting is the largest mortality factor in black bears in the Commonwealth (Ternent, 2006). Annual total harvests in Pennsylvania over the last 5 years (2015-2019) have averaged 3,701 bears per year (range: 3,153 to 4,653; Pennsylvania Game Commission, 2021). Hunting accounted for 48% of male deaths and 37% of female deaths in all bears over two years of age between 1979-1996 (Diefenbach & Alt, 1998). Vehicle collisions account for the second largest cause of bear mortality, representing approximately 10% of all deaths (Ternent, 2006). Other human-associated mortality factors include poaching and nuisance removal for property damage or threatening interactions. Together, these other factors only account for about 59 deaths per year, or 0.5% from 1995-2010. Historically, black bear mortality in Pennsylvania not directly associated with human interactions have been less common, but include intraspecies aggression, starvation, and disease.

Wildlife Diseases

Relative to hunting and other human-associated causes of mortality, disease has historically been an uncommon source of morbidity and mortality in black bears. However, as the bear population grows and geographically expands, risk factors for infectious and non-infectious diseases could potentially change due to increasing bear density and emergence of new interfaces between bears and humans, domestic animals, and other wildlife. Consequently, a critical component of managing the thriving black bear population in Pennsylvania is monitoring the occurrence of disease. Disease is defined as an impairment of normal functioning or state of an

animal, often associated with clinical signs or lesions (Merriam-Webster, 2021). The cause of disease (i.e. etiology) can be infectious or non-infectious (Merriam-Webster, 2021). A pathogen is an organism that causes or contributes to an infectious disease (Merriam-Webster, 2021), and includes viruses, bacteria, parasites, or fungi. Most wildlife diseases are multifactorial, involving complex interactions of the host, agent, and environment. Consequently, it is important to recognize that disease, from infectious or non-infectious etiologies, may result in a spectrum of presentations ranging from no overt signs or lesions to severe disease and mortality.

To account for the complexities of disease ecology and presentation (in individual animals or a population), effective surveillance programs must employ a multifaceted approach to identify and monitor pathogens and diseases in wildlife populations, including active and passive surveillance approaches. Passive surveillance includes postmortem examination of an animal to determine the cause and nature of the disease (Stallknecht, 2007). In most wildlife agencies, this includes diagnostic investigations of morbidity and mortality events. Although passive surveillance can provide insight into diseases that occur in a species or population, the data is entirely dependent on mortality events being identified, cases being submitted, and the quality of carcasses being maintained (minimize decomposition or scavenging). Since this approach is dependent on detection of morbidity and mortality, it is not a good method to identify diseases that cause mild or asymptomatic infections. Also, since the mortality cannot be predicted or controlled, this approach will not necessarily yield complete geographic or host demographic representation of the population.

Active surveillance can serve as an effective complement to passive surveillance in wildlife (MacDonald et al., 2020). Active surveillance involves targeted testing of an animal or

population for pathogens/etiologies or diseases (Artois et al., 2009). Active surveillance enables infections to be detected even without the outward signs of disease, thus allowing for a more accurate determination of pathogens or etiologies within a population. It also allows for more detailed analyses to be performed, such as determination of prevalence or incidence. Finally, depending on how samples are collected, active surveillance also allows for potentially more control on where, when, and who the samples are collected from; addressing questions that may remain unanswered through passive surveillance. One limitation of active surveillance is that it may only identify the presence of a pathogen/etiology and not necessarily provide information on associated disease. For example, surveillance for antibodies to a pathogen may indicate exposure to that pathogen in the population, but it will not provide any data on occurrence of disease. Because of these limitations for each approach, active and passive surveillance can be used in concert to effectively identify and research both pathogens/etiologies and disease within a wildlife population (MacDonald et al., 2020).

Diseases in Black Bears

Black bears are susceptible to a wide diversity of both infectious and non-infectious diseases. In addition to trauma, non-infectious diseases reported in bears include toxicosis, nutritional diseases, congenital malformations, dental disease, endocrine disorders, degenerative joint disease, and neoplasia (Bourne et al., 2010). These diseases are generally uncommon in bears, especially wild black bears, though some may occur more frequently in captivity, associated with husbandry or geriatric diseases.

Infectious diseases are a regular cause of mortality in black bears and have been associated with a plethora of pathogens, including viruses, bacteria, fungi, or parasites. Globally, infectious diseases reported in black bears include tuberculosis, Lyme disease, rabies, infectious hepatitis, and leptospirosis; most of these are asymptomatic infections without outward signs of disease or case reports (Di Salvo & Chomel, 2020; Mortenson, 1998; Roberts et al., 2009; Stephenson et al., 2015). Of these pathogens, parasitic diseases appear to be the most common cause of morbidity and mortality, particularly in the Mid-Atlantic and Northeast, where mange is an increasingly common disease (Bourne et al., 2010; Mortenson, 1998; Niedringhaus et al., 2019).

Although a diversity of diseases have been reported in American black bears, none appear to be population limiting throughout their range in North America. However, this does not negate the importance of identifying and monitoring black bear diseases in Pennsylvania, as the prevalence, manifestations, and/or impacts of diseases/pathogens could change with increasing and expanding populations. Beyond the direct population impacts, there are additional reasons to study pathogens and diseases of black bears, including potential health implications for other wildlife, humans, and/or domestic animals and improved communication with hunters or the general public that may have questions regarding diseases in black bears. Current data on diseases in black bears in North America are largely restricted to sporadic case reports and research focused on a relatively few diseases associated with regional outbreaks (e.g. mange). This has resulted in a relative dearth of information on black bear pathogens and disease. Consequently, a first step in building a more robust understanding of black bear health and disease involves defining what pathogens and diseases exist within this species.

Parasites of Black Bears

A parasite is an organism that lives on or within another organism and relies on the host for survival (Merriam Webster, 2021;Roberts et al., 2009). Parasites can be divided into two broad categories based on the location of infestation: endoparasites and ectoparasites. Endoparasites live internally within the host, while ectoparasites live externally on the surface of the host (Roberts et al., 2009). In addition, parasites are divided into five broad classifications based on morphology: protozoa, nematodes, cestodes, trematodes, and arthropods (Garcia, 2009). These classifications are further broken down into categories based on morphology, genetics, and location of infection.

Protozoa are single-celled organisms that have a wide range of structural diversity and complexity (Roberts et al., 2009). Protozoa can be found in various tissues or bodily fluids, including the intestines, blood, or other visceral organs (Garcia, 2009). Examples of protozoa reported from black bears include *Eimeria albertensis* and *E. borealis* (Rogers & Rogers). Nematodes, often called roundworms, are common endoparasites that are bilaterally symmetrical and elongated, and have tapered ends and a pseudocoel (Roberts et al., 2009). Nematodes can be free-living or located in a wide diversity of tissues or bodily fluids, including the intestines, skin, or blood. Examples of nematodes reported in bears include *Dirofilaria* spp. and *Trichinella* spp. (Garcia, 2009). Cestodes, commonly known as tapeworms, are multicellular endoparasites that often have complex life cycles, involving multiple hosts. Their bodies consist of scolex (head), neck, and strobila (variably-sized chain of segments called proglottids) (Roberts et al., 2009). Most adult cestodes are present in the gastrointestinal tract, but larval forms can be found in multiple tissues. Examples of cestodes reported in black bears are *Taenia* spp., such as *T.*

pisiformis and *T. saginata* (Rogers & Rogers). Trematodes, also called flukes, are some of the most common parasites as they can infect every class of vertebrate (Roberts et al., 2009). They are dorsoventrally flattened and oval-shaped and have an oral sucker. Depending on the species, trematodes can inhabit a diversity of tissues and organs (Roberts et al., 2009), but are most commonly found in the intestines, lungs, liver, and blood (Garcia, 2009). An example of a trematode reported in black bears is *Neorickettsia helminthoeca* (Rogers & Rogers). Lastly, arthropods are multicellular organisms that are segmented and have a chitinous exoskeleton (Roberts et al., 2009). They can act as the definitive host, intermediate host, or vector. They are found out in the environment or within other organisms. Examples of arthropods from black bears include *Dermacentor andersoni* (tick) and *Sarcoptes scabiei* (mite) (Rogers & Rogers).

A wide diversity of parasites have been reported within black bears, including nematodes, arthropods, cestodes, trematodes, and protozoa (Rogers & Rogers). However, not all of these parasite infestations in bears are associated with overt disease, and many are harbored asymptomatically. Notable parasites that have been associated with disease in wild black bears include multiple species of mites associated with mange, including *Sarcoptes scabiei*, *Demodex ursi*, and *Ursicoptes americanus* (Rogers & Rogers). Of these, mange is the most common non-human associated cause of morbidity in black bears in Pennsylvania.

Mange in Black Bears

Mange is a general term referring to a parasitic disease of the skin caused by mites. Mites are microscopic arthropods that can burrow through the skin and/or live within the hair follicles of vertebrates (Roberts et al., 2009). Species of mites are differentiated based on morphology,

including body shape, exoskeleton, and color. Mange can occur in most animals, including humans; however, the disease presentation (signs and lesions) and causative mite vary by host species.

Three species of mites have reportedly been associated with mange in black bears: *Sarcoptes scabiei*, *Demodex ursi*, and *Ursicoptes americanus* (Niedringhaus et al., 2019). Each of these mite species vary in their biology, transmission, and host range. *Sarcoptes scabiei* is globally distributed and can infest a wide range of mammal hosts, including humans. In North America, bears and canids (e.g. wolves, coyotes, and red foxes) are the most common wildlife species infested with *S. scabiei* (Niedringhaus et al. 2019). It is theorized that there are multiple species-specific variants of *S. scabiei*. These species-variants can infest other host animals, but do not complete their lifecycle or have sustainable infestations unless they are on their specific host species (Arlian et al., 1984). *Sarcoptes scabiei* causes the disease sarcoptic mange by burrowing into the epidermis and feeding on epithelial cells of the skin and lymph. It has five life stages: egg, larva, protonymph, tritonymph, and adult, all which reside on the same host animal. Adult females penetrate the skin of the host, creating burrows, where eggs will be laid. The eggs will then develop to larvae, then to nymph, and eventually adult (Niedringhaus et al. 2019). The entire life cycle takes approximately 2 weeks to complete. Transmission of *S. scabiei* can occur through direct contact between an infected and non-infected host. Although the mite is not highly persistent in the environment, and its tenacity is temperature dependent, *S. scabiei* can survive for days off the host to allow for indirect transmission as well (Niedringhaus et al. 2019).

Demodex ursi is a mite that infests hair follicles and is specific to bears. The life cycle of *Demodex ursi* is similar to other Demodex species. There are 5 life stages of the mite: egg, larva,

protonymph, tritonymph, and adult; all stages occur within the host animal (Saari et al., 2019). Adults reproduce within the skin and live within the hair follicles. The life cycle takes approximately 3-4 weeks. Infestation leading to disease is called demodicosis or demodectic mange (Desch, 2009).

Ursicoptes americanus is another mite that burrows through the epidermis and is specific to bears. The disease associated with this mite is audycoptic mange. Little is known about this form of mange in bears or the life cycle of the mite. It has been reported in captive bears in Kansas and wild black bears in Idaho, Virginia, and Pennsylvania. (Yunker et al., 1980).

Although these mites differ in host range, biology, and pathogenesis, their associated syndromes are impossible to definitively distinguish based on gross lesions alone, which include varying degrees of alopecia, crusting, fissuring, and hyperpigmentation of the skin. Consequently, ancillary diagnostics (e.g. skin scrapes and molecular assays) are required to identify the mite associated with mange in black bears. Confirming the cause of mange is important, as each of these mites have different implications for bears, other wildlife, humans, and domestic animals, and may require different management approaches.

Historically, all three types of mange in black bears were uncommon and sporadic diseases that most often involved individual animals. Starting the early 1990s, sarcoptic mange has become increasingly more common in the Mid-Atlantic and Northeast. Sarcoptic mange was initially detected in black bears with mange in the central-western area of Pennsylvania in 1991(Sommerer, 2014). Since this detection, sarcoptic mange has spread throughout much of the Commonwealth and into adjacent states of New York, Maryland, Virginia, and West Virginia

(Niedringhaus et al., 2019). It is now the most common non-human source of morbidity/mortality in Pennsylvania bears.

Sarcoptic mange in black bears results in variable, but often severe, skin disease, including alopecia, hyperkeratosis, and erythema; skin thickening, crusting, and fissuring; and secondary bacteria and yeast infections (Niedringhaus et al., 2019). In severe cases, most of the skin covering the body is affected (>90%). Early in infestation, bears experience severe pruritus and will be observed incessantly itching; however, as disease progresses in severity, the animals become emaciated, weak, lethargic, and unaware of their surroundings (Niedringhaus et al., 2019). Later in infection, lesions can be a result of a hypersensitivity response rather than direct damage from the mites. The type and extent of response is dependent on the host species and immune status of the host (Pence & Ueckermann, 2002). Sarcoptic mange in black bears can be a fatal disease, but the case mortality rate is currently unknown.

The origins and risk factors for the sarcoptic mange epidemic in black bears in the Mid-Atlantic and Northeast are currently unknown. One theory is that the increasing sarcoptic mange cases is related to the increasing and expanding bear population. In this theory, as black bear populations increase and expand, so do sarcoptic mange cases. Collectively, the existing data on sarcoptic mange indicates that this theory is unlikely. Interestingly, some of the highest density of black bear populations in Northeast Pennsylvania and New Jersey currently have little to no reports of sarcoptic mange (Niedringhaus et al., 2019). The other common theory is that a bear-adapted strain of *S. scabiei* emerged in Central Pennsylvania around the 1990s (or before) that has expanded outward from that focus. Both of these theories are the subject of active research projects. While the mechanism of disease emergence and spread is unknown, it is established

that sarcoptic mange is endemic in black bears throughout much of their range in Pennsylvania and adjacent states. In these areas, sarcoptic mange is a common cause of morbidity. Despite this high prevalence and endemicity, there is no evidence that sarcoptic mange is having a significant negative population impact in Pennsylvania. Many areas where sarcoptic mange is common continue to have increasing bear populations at similar rates to areas where mange does not exist, and relatively high levels of reproduction (Pennsylvania Game Commission, 2021).

Nematodes in Black Bears

Nematodes are common endo and ectoparasites of black bears. Also referred to as roundworms, nematodes are elongated and bilaterally symmetrical with tapered ends (Roberts et al., 2009). They typically have an outer cuticle covering their body, a complete digestive system, longitudinally-arranged muscles, and no flagella or cilia. Adult nematodes can vary in size from about 0.5 millimeters to 10 meters in length. Males and females are morphologically distinct; females are typically larger than males and have a curled tail (Roberts et al., 2009).

Based on morphology and host and tissue tropism, nematodes are separated into Class *Chromadorea* or Class *Enoplea*, and then further divided eventually into different species (Roberts et al., 2009). Nematodes exhibit a wide diversity of tissue tropisms, including intestinal tract (e.g. *Trichostrongylus* spp.) or blood, bodily fluids, and skin/skin capillaries (e.g. *Dirofilaria* spp.) (Garcia, 2009). Associated with this tropism, nematodes also exhibit a wide-diversity of transmission mechanisms, including some that are vector-borne and others that utilize a fecal-oral route.

Multiple species of nematodes have been reported from black bears, including *Baylisascaris transfuga, Baylisascaris multipapillata, Ascaridoids, Uncinaria yukonensis, Crenosoma, Gongylonema pulchrum, Dirofilaria ursi*, and *Trichinella spiralis* (Rogers & Rogers). None of these are regularly associated with overt clinical signs or lesions in wild black bears; they typically result in asymptomatic infections. However, due to their grossly-visible size and potential for large worm burdens, nematodes are frequently identified by hunters during field dressing and are a common cause for questions posed to wildlife agency personnel.

Filarial Nematodes of Black Bears

Filarial nematodes are a common parasite in mammals and birds. In general, the adult worms are slender and have a reduced buccal capsule and lips (Roberts et al., 2009). All species of filarial worms utilize arthropods (e.g. mosquitoes and black flies) as an intermediate host. As with most nematodes, females are much larger than males. Adult filarial worms occupy specific tissues of their hosts, that vary with filarial species, and produce microfilaria through sexual reproduction (Samuel et al., 2001). Microfilariae circulate within the host's blood and are ingested by blood-feeding arthropod vectors where they develop into third stage larvae (L3). The L3 larvae then migrate to the mouth area of the vector (specific area depending on the vector) where they can be transmitted to another vertebrate host through vector feeding. Once in the new host, the larvae will undergo two molts (L4 larvae) and migrate to the specific tissues of preference and become adults. Within the life cycle of filarial nematodes, the arthropods serve as an intermediate host and are biological vectors.

Different filarial worm species exhibit varying host tropism and range (Samuel et al., 2001). For example, *Brugia malayi* has a wide host range; it can infect a variety of animals including humans, monkeys, canids, felids, viverrids, and pangolins. Alternatively, *Monanema martini* has a narrow host range, and predominantly has been reported in African murid rodents (Samuel et al., 2001). This host specificity results in some filarial worms being unable to survive and reproduce in an aberrant host. In aberrant hosts, filarial larvae may produce infections, but the larvae do not develop into later life stages or adults. Because of this, microfilaria are not produced at significant levels to allow for infection of the blood feeding vector, and these aberrant hosts are thus considered "dead-ends" in regards to transmission.

Filarial worms have been reported in a diversity of animals, including wildlife, domestic animals, and humans. While infections with many of these do not result in clinical disease or lesions, there are some notable exceptions, including *Wuchereria bancrofti* causing elephantiasis in humans, *Onchocerca volvulus* causing onchocerciasis in humans, and *Dirofilaria immitis* causing heartworm disease in dogs (Roberts et al., 2009). In these cases, disease is associated with the direct damage to host tissue from the nematode and/or inflammation/immune response to the adult worms and/or the microfilaria.

Filarial worms have been reported in black bears, including *Dirofilaria immitis* and *Dirofilaria ursi*. To date, most of these infections are not associated with overt disease in black bears.

Dirofilaria

Dirofilaria is a genus of filarial nematodes that are common in a wide range of domestic and wild animals. The tissue tropism for adult worms varies between *Dirofilaria* species but are most often found within the subcutaneous tissues or occasionally pulmonary artery of its host; Microfilaria are the larval stages that are produced by adult worms through sexual reproduction and circulate in the blood (Bowman & Atkins, 2009). Microfilariae of *Dirofilaria* are small, unsheathed, and have tapered tails. The *Dirofilaria* genus has 27 species (Michalski et al., 2010) including: *D. acutiuscula, D. ailure, D. bonnei, D. cancrivori, D. corynodes, D. freitasi, D. genettae, D. granulosa, D. immitis, D. incrassata, D. linstowi, D. lutrae, D. macacae, D. macrodemos, D. magnilarvatum, D. minor, D. pagumae, D. panamensis, D. repens, D. sachsi, D. spectans, D. striata, D. subdermata, D. sudanensis, D. tawila, D. tenuis, D. ursi* (Canestri Trotti et al., 1997). Of these, the most significant species for animal health are *D. immitis* in canids (Bowman & Atkins, 2009), and *D. repens* in canids and felids (Poppert et al., 2009). Each *Dirofilaria* species exhibits varying host tropism and range, as well as biological vectors. Some species are considered zoonotic (Table 1); however, the transmissibility for humans is often rare, and humans are considered a “dead end” host. Most of these human infections present themselves as tumors or abscesses in subcutaneous tissues associated with the eyes, breast, and upper lip (Samuel et al., 2001).

Table 1. *Dirofilaria* species that have been reported in humans.

Species	Adult Tropism	Definitive Host	Vector	Distribution	Infection Rates in Animals	Citations
D. immitis	Pulmonary	Dogs	Mosquito	United States and warm climates globally	Common	Bowman & Atkins, 200
D. repens	Subcutaneous	Wild and domestic carnivores	Mosquito	Europe, Africa, Asia	Common	Poppert et al., 2009
D. striata	Subcutaneous	Felids	Mosquito	United States	Rare	Wyatt et al., 2020
D. tenuis	Subcutaneous	Racoons	Mosquito	Southeast United States	Common	Vincent et al., 2013
D. ursi	Subcutaneous	Bears	Black Fly	Northern United States, Canada, and Japan	Rare	Michalski et al., 2010

Dirofilaria ursi

Dirofilaria ursi is a filarial nematode reported from bears, including the American black bears, grizzly bears (*Ursus arctos*), and Asiatic black bears (*Ursus thibetanus*) (Gywnn et al., 2017; Yokohata et al., 1990). Its host range is typically restricted to bears, but there have been sporadic reports of human infections with *D. ursi*-like nematodes. *Dirofilaria ursi* is vectored through the bite of black flies (*Simuliidae*). The adult stages of this nematode reside within the subcutaneous tissues throughout the body or connective tissues surrounding organs of the thoracic and abdominal cavities of the host (Michalski et al., 2010). Microfilaria are produced through sexual reproduction, circulate in the blood, and are ingested by black flies when they take a blood meal from an infected bear. In black flies, the microfilaria develop to L3 larvae in the malpighian tubules. The larvae then migrate to the mouth of the black fly and are transmitted to the next host, where they develop into later larvae stages and then adults to complete the lifecycle (Michalski et al., 2010). The duration of the *D. ursi* life cycle is currently unknown; however, comparable *Dirofilaria* spp. such as *D. repens* mature in about six months (Poppert et al., 2009).

None of the *D. ursi* infections identified in bears to date have been associated with overt clinical signs or significant lesions. Consequently, surveillance for this parasite relies on active screening for adult nematodes in the subcutaneous or connective tissues, internal organs, and/or microfilaria in the blood. Due to marked variability in where adult worms can be located, most recent active surveys have relied on detection of microfilariae in blood. Morphologically, microfilaria of *Dirofilaria* spp. are identified by being unsheathed and having tapered tails

(Michalski et al., 2010). Further speciation is challenging, and most contemporary surveys utilize molecular techniques and sequencing (Gywnn et al., 2017).

Dirofilaria ursi has been reported in black bears, grizzly bears, and Asiatic black bears from Wisconsin, Minnesota, and Michigan, in the United States; New Brunswick, the Province of Quebec, Alberta, and British Columbia in Canada; and Japan (Catalano et al., 2015; Duffy et al., 1994; Frechette & Rau, 1977; Manville, 1978; Michalski et al., 2010; Rogers, 1975; Uni, 1983; Yokohata et al., 1990). One of these identifications consisted of a case report involving an Asiatic black bear that was shot in Kyushu, Japan, and necropsied; *Dirofilaria ursi* was identified in the tracheal and esophageal connective tissues (Yokohata et al., 1990). Larger surveys for *D. ursi* in black bears from North America have been conducted in multiple states or Canadian provinces, either by screening for adult filarial nematodes or microfilaria (Catalano et al., 2015; Duffy et al., 1994; Frechette & Rau, 1977; Manville, 1978; Michalski et al., 2010; Rogers, 1975; Uni, 1983).

Reported prevalence of infection with adult *D. ursi* has been highly variable, ranging from 7-57%. During 1989-1991, adult *D. ursi* were identified in the perirenal and other connective tissues of 37/110 (33.7% prevalence) hunter-harvested, road-killed, or nuisance-killed black bears from New Brunswick, Canada (Duffy et al., 1994). In Alberta and British Columbia, Canada, adult *D. ursi* were observed in the subcutaneous tissues, peri-tracheal fascia, and/or peritoneal fascia of 7/22 (31.8% prevalence) black bear and 2/7 (28.6% prevalence) grizzly bear carcasses that were collected and examined during 2015 (Catalano et al., 2015). Adult *D. ursi* were also found within the superficial abdominal fascia and adipose tissue in the inguinal region

of 12/21 (57% prevalence) black bear carcasses killed on or near Quebec parks from the Province of Quebec, Canada during 1971-1972 (Frechette & Rau, 1977).

During the fall of 2010, microfilaria consistent with *D. ursi* were found in 10/47 (21% prevalence) blood smears from hunter-harvested black bears from northern Wisconsin, USA, and confirmed by polymerase chain reaction (Michalski et al., 2010). Previous *Dirofilaria* spp. surveys were conducted in hunter-harvested black bears in northern Wisconsin between 1974-1975, which detected microfilaria in the blood of 17/90 bears (19% prevalence). During this earlier study, black bear carcasses were also examined for adult *D. ursi*. Adult nematodes were identified in a total of 2/28 (7% prevalence) hunter-harvested black bears, which were located in connective tissues surrounding the aorta, kidneys, and rectum (Manville, 1978). This study highlights some of the challenges of conducting surveillance for *D. ursi* in black bears. Microfilaria are easier to detect in peripheral blood smears, but the infection must be productive to have circulating microfilaria. Another disadvantage of examining blood smears is that species of *Dirofilaria* cannot be distinguished based on morphology of microfilaria alone. Conducting surveillance by identifying adult stages is challenging because of the wide range of locations in the body where the adults can reside. Consequently, the adult worms may easily be missed and prevalence underestimated.

Although rare, human infections have been reported with *D. ursi*-like parasites, including isolated cases in Vermont, Florida, Canada, and Japan (Beaver et al., 1987; Haldane et al., 1996; Herzberg et al., 1995; Yamada et al., 2017). Reported symptoms included nodules on the skin that were warm and tender, fever, diarrhea, and vomiting. In these cases, larval and/or adult worms were present in the nodules and were identified as "*D. ursi*-like" based on morphology

(i.e. longitudinal cuticular ridges regularly and widely spaced on the outer surface) (Beaver et al., 1987; Haldane et al, 1996; Herzberg et al. 1995), or a combination of morphology and molecular diagnostics (Yamada et al., 2017). Besides the infections in bears and humans, there have been no *D. ursi* infections reported in any other animals.

Filarial nematodes, including *D. ursi*, have been found to be infected with the intracellular bacteria *Wolbachia*. The presence of *Wolbachia* in *D. ursi* was confirmed through immunohistochemistry and polymerase chain reaction (PCR), although specific species of *Wolbachia* were not reported (Michalski et al., 2010). In other nematodes, *Wolbachia* plays an important role in filarial worm survival and disease pathogenesis (Roberts et al., 2009). In *D. ursi*, *Wolbachia* has been identified within the hypodermic and lateral cord of a female specimen (Michalski et al., 2010). Currently, the relationship between *D. ursi* and *Wolbachia* is poorly understood, and additional research is warranted. However, previous studies have proposed that *Wolbachia* may contribute to the overall health and reproductive capacity of *D. ursi* (Michalski et al., 2010; Roberts et al., 2009).

Conclusion

Black bear populations in Pennsylvania have been increasing and expanding over the past 40 years. As the population continues to increase, it is critical to monitor indicators that bears are reaching a biological or wildlife acceptance capacity, which could limit their growth directly or indirectly. Disease is an important measure of a wildlife population exceeding the biological carrying capacity; however, there currently is a dearth of information on diseases and pathogens of black bears in Pennsylvania. The results of this literature review indicate that black bears in

North America experience morbidity and mortality associated with a wide diversity of infectious and non-infectious diseases, but all of these are rare relative to human associated mortality. In addition, black bears harbor a plethora of pathogens in the absence of overt disease, including viruses, parasites, and bacteria. None of these diseases or pathogens currently appear to be limiting black bear populations in North America. However, with the increasing and expanding black bear population in Pennsylvania and the emerging disease of sarcoptic mange, it is critical to define the diseases and pathogens of black bears in the Commonwealth now, and continue to monitor these moving forward.

CHAPTER 1: INTRODUCTION

Dirofilaria ursi is a filarial nematode of bears, including American black bears (*Ursus americanus*), grizzly bears (*Ursus arctos*), and Asiatic black bears (*Ursus thibetanus*) (Gywnn et al., 2017; Yokohata et al., 1990). Adult worms reside within the subcutis and the connective tissues of the thoracic and abdominal cavities (Michalski et al., 2010). Larval stages, referred to as microfilaria, are produced through sexual reproduction and circulate in the blood. Microfilaria are ingested by black flies (*Simuliidae*) when they obtain a blood meal from an infected black bear, and subsequently develop to L3 larva within the vector prior to being transmitted to the next host. Once inoculated into the new host, the larvae will continue to develop and mature until the adult stage is reached (Michalski et al., 2010).

Dirofilaria ursi have been identified sporadically in bears throughout Canada, Japan, and the northern United States (Catalano et al., 2015; Duffy et al., 1994; Frechette & Rau, 1977; Manville, 1978; Michalski et al., 2010; Rogers, 1975; Uni, 1983; Yokohata et al., 1990). Surveillance for *D. ursi* can focus on detection of adult worms in tissues or microfilaria on blood films. Each of these approaches have different advantages or disadvantages. Adult worms can be used to identify species morphologically and can identify infections that are not producing microfilaria (i.e. single sex adult nematodes). However, adult worms vary in their anatomic tropism and, consequently, can be easily missed. Microfilaria are easier to detect, but the species may not be identified based on morphology alone. Reported prevalence estimates from black bears in North America based on detection of adults include: 33.7 % (37/110) in New Brunswick, Canada during 1989-1991 (Duffy et al., 1994); 57% (12/21) in the Province of Quebec, Canada during 1971-1972 (Frechette & Rau, 1977); 31.8% (7/22) in Alberta and British

Columbia, Canada during 2015 (Catalano et al., 2015); and 7% (2/28) in northern Wisconsin during 1974-1975 (Manville, 1978). Reported prevalence estimates in black bears in North America based on detection of microfilaria include: 19% (17/90) in northern Wisconsin during 1974-1975 (Manville, 1978); and 21% (10/47) in northwestern Wisconsin during 2010 (Michalski et al., 2010). *Dirofilaria ursi* infections have not been associated with overt disease (i.e. clinical signs or lesions) in bears. Although other species of *Dirofilaria* can exhibit a wide host range, *D. ursi* appears to be relatively restricted, with infections only being confirmed in bears. In addition, however, there have been sporadic reports of adult *D. ursi*-like worms associated with skin nodules in humans (Beaver et al., 1987; Haldane et al., 1996; Herzberg et al., 1995; Yamada et al., 2017). While extremely rare and unconfirmed as *D. ursi*, these human cases suggest additional research is warranted.

Although black bears were once common throughout much of Pennsylvania, their populations experienced a dramatic decline during the period of European settlement in the 1800s, as a result of over-hunting and conversion of forested habitat to agricultural lands (Ternent, 2006). For much of the 20th century, black bear populations in the Commonwealth remained below 4,000 bears statewide and were largely restricted to the Northcentral and Northeast parts of the state. As a result of active habitat restoration and the establishment of a bear management program (e.g. hunting regulations, population monitoring), black bear populations in Pennsylvania have steadily increased and expanded since the early 1980s. Currently, there are estimated to be 18,000 bears in Pennsylvania that are distributed throughout much of the state (Ternent, 2019). While this is a wildlife management success story, with important benefits, there are also potential negative aspects to the expanding bear population, such as negative bear-human or bear-domestic animal interactions and/or disease.

Due to increasing bear density and interfaces with humans, domestic animals, and other wildlife, the potential exists for diseases in black bears to emerge or change in virulence or prevalence. Consequently, an important component of the managing bears in Pennsylvania is monitoring health, disease, and pathogens harbored by black bears. Currently, there is a relative dearth of surveillance data on diseases and pathogens of black bears in the Commonwealth. The objective of this research is to survey black bears in Pennsylvania for microfilaria of *Dirofilari*a spp. We hypothesize that microfilaria will be detected in black bears from Pennsylvania at prevalence estimates comparable to existing surveillance data from previous studies in the upper Midwest (19%-21%) (Manville, 1978; Michalski et al., 2010).

CHAPTER 2: MATERIALS AND METHODS

Sample Collection

During 2018-2020, black bears in Pennsylvania were screened for *Dirofilaria* spp. through the examination of peripheral blood smears for microfilaria. Blood samples were collected from black bears by Pennsylvania Game Commission employees or Penn State University graduate students for the purposes of multiple other ongoing research projects or agency management activities. This sampling effort was approved by the Pennsylvania Game Commission (state collection permit ID# 42115) and an Institutional Animal Care and Use Committee at Penn State University (ID# 47978). Black bears were sampled at multiple times during the year and through multiple mechanisms, including trapped bears in the summer (July-September), hunter-harvested bears in the fall (October-December), collared adult sows and their cubs/yearlings during the winter while in their dens (January-March), and any opportunistic sampling of diagnostic cases and nuisance removals during the spring (April-June).

During the summer, black bears were trapped by Pennsylvania Game Commission personnel using baited culvert traps. This is part of a state-wide program conducted each year to estimate black bear populations in Pennsylvania. Black bears in the trap were anesthetized using a mixture of Ketamine-Xylazine. Ketamine was administered at 1 mL per 45.5 kg body weight (4.4 mg/kg), and Xylazine was administered at 1 mL per 111.1 kg body weight (1.8 mg/kg). The dosing protocols were standard protocols used by the Pennsylvania Game Commission. After immobilization, bears were given a brief physical exam, ear-tagged, and a sample was collected

via the femoral vein. Blood samples were immediately transferred to a serum-separator tube and a purple-top vacutainer tube containing the anticoagulant Potassium EDTA (BD Vacutainer® New Jersey, USA). Both tubes were placed on ice packs in the field until they were delivered to a laboratory/office for processing.

During November and early December, blood samples were collected from recently hunter-harvested black bears (< 24 hours) at check stations in Pennsylvania. Briefly, visible blood was scooped out of the body cavity of field dressed bears using polypropylene centrifuge tubes (Thermo Fisher ScientificTM, Waltham, Massachusetts, USA). The blood was transferred to serum separator tubes and purple-top Potassium EDTA vacutainer tubes at the check station. Serum separator tubes were centrifuged immediately at the check station and transferred to microcentrifuge tubes. The purple-top vacutainer and microcentrifuge tubes were stored on ice packs in the field until they were delivered to the laboratory/office for further processing.

Blood samples were collected from collared sows and their offspring (either cubs or yearlings) during the winter while in their den. Dens were visited, and bears were handled by Pennsylvania Game Commission personnel as part of a long-term monitoring study on black bear reproduction. Yearling dens were visited in February, and cub dens were visited in March. Briefly, dens with radio- or GPS-collared sows were located, and the sow was anesthetized using the Ketamine-Xylazine cocktail described above. If it was a yearling den, yearlings were anesthetized as well, if possible. Cubs were not anesthetized and were handled without chemical sedation. Sows and yearlings or cubs were given a physical exam, ear-tagged (if not already), and a blood sample was collected from the femoral vein (sows and yearlings) or jugular vein (cubs). Blood was immediately placed into serum separator tubes and purple-top Potassium

EDTA vacutainer tubes in the field. Both tubes were placed on ice packs in the field until they were delivered back to the laboratory that evening and processed.

Data on age, sex, season, county, date, and presence or absence of sarcoptic mange were collected from all bears that were sampled. Age was defined as adults or juveniles (cubs and yearlings) based on dentition. Season was categorized as follows: January to March was winter, April to June was spring, July to September was summer, and October to December was fall. Presence of mange was determined based on consistent gross lesions and confirmation of *S. scabiei* on skin scrapes.

Sample Processing

For trapped or denned bears, serum separator tubes were centrifuged, and the serum was transferred to microcentrifuge tubes once back at the laboratory/office. All microcentrifuge tubes were stored at -20º C in the Pennsylvania Game Commission serum repository. The purple-top vacutainer tubes were submitted to Animal Resources Clinical Pathology Laboratory at Penn State University (University Park, Pennsylvania, USA) for a Complete Blood Count (CBC) using the ProCyte Dx (IDEXX, Westbrook, Maine, USA). Prior to conducting the CBC, blood smears were prepared from the anticoagulated blood samples and manually stained with a Romanowsky-type stain (Diff-Quik).

Blood smears were examined blindly by a veterinarian and undergraduate research student with a light microscope using the 10x objective for any worm-like organisms. Any suspect worms were further examined with the 40x objective to identify morphologic features consistent

with *Dirofilaria* spp. including unsheathed microfilaria of appropriate length and width that were filled with multiple closely packed nuclei. The number of microfilaria were counted on each slide and recorded as “number of microfilaria per blood smear”. All positive blood smears were sent to the University of Georgia for confirmation by a parasitologist, where the microfilaria were identified as *Dirofilaria* spp. based on published morphologic features (Michalski et al., 2010).

Statistical Analysis

A binary logistic regression model was used to evaluate the association between microfilaria (+ or -) on blood smears and a number of variables, including sex, age, weight, presence of mange, and season. Season was not able to be evaluated by a binary logistic regression model because there were no positives in the 8 samples collected during the winter season. Consequently, the 8 negative winter data points were removed from the data in order to evaluate spring, summer, and fall using a separate binary logistic regression model (with the seasonal variable included). When individual bears were sampled on multiple dates during the study period (typically GPS collared research bears), only the first sampling date during this period was used for analyses. An alpha level of 0.05 was used to determine significance for all analyses.

CHAPTER 3: RESULTS

During 2018-2020, 129 blood samples were collected and examined for microfilaria. Of these samples, 33/129 (25.6%) contained microfilaria. The mean number of microfilaria per blood smear was 27.4 (range: 1 to 282). Annual percentage of positives over the study period were as follows: 8/40 (20.0%) during 2018, 14/46 (30.4%) during 2019, and 11/43 (25.6%) during 2020. Other than the bears with mange, none of the bears positive for microfilaria exhibited any clinical signs of disease or had any significant lesions that could be attributed to *Dirofilaria* spp.

Summaries of age, sex, body weight, and season distribution relative to positive or negative *Dirofilaria* spp. results are presented in Tables 2 to 5. None of these variables had a significant effect on black bears being positive for microfilaria (Table 6). Of the positives, 60.6% were females (20/33), and 39.4% were males (13/33). The majority of positives (31/33; 93.9%) were from adult bears, with one being a juvenile and one being of unknown age. The weights of positive bears ranged from 43 pounds to 327 pounds, with the average of 118.6 pounds. Of the positive bears, 9/33 (27.3%) reportedly had sarcoptic mange. Microfilaria were identified in three of the four seasons; 42.4% (14/33) of the positives were from samples collected in the spring, 45.5% (15/33) from summer, 9.1% (4/33) from fall, and 0% (0/8) from winter.

Sex, age, season, and weight were not significantly associated with black bears being positive for microfilaria (Table 6). Black bears that had sarcoptic mange were significantly less likely to be positive for microfilaria than bears without mange (p-values = 0.039 (season variable excluded) and 0.033 (season variable included without winter)). When all season data was

excluded, 18.0% of bears with mange were positive for microfilaria, while 30.4% of bears without mange were positive for microfilaria (Figure 1). When spring, summer, and fall season results were also included, 19.1% of bears with mange also tested positive for microfilaria, while 32.4% of bears without mange were positive for microfilaria (Figure 2).

Table 2. Sex distribution for black bear blood smears collected from Pennsylvania that were examined for *Dirofilaria* spp., 2018-2020. Sex was not significantly associated with black bears being positive for microfilaria on blood smears.

Sex	Total Number in Samples	Total Number of Positives	Percent Positive
Male	50	13	26.0%
Female	79	20	25.3%

Table 3. Age distribution for black bear blood smears collected from Pennsylvania that were examined for *Dirofilaria* spp., 2018-2020. Age was determined by dentition and categories as adult or juvenile (yearling or cub). Age was not significantly associated with black bears being positive for microfilaria on blood smears

Age	Total Number in Samples	Total Number of Positives	Percent Positive
Juvenile	5	1	20.0%
Adult	124	31	25.0%
Unknown	1	1	-

Table 4. Association between confirmed sarcoptic mange in black bears from Pennsylvania and presence of *Dirofilaria* spp. on blood smears, 2018-2020. Mange was significantly associated with black bears being positive for microfilaria on blood smears

Mange Presence	Total Number in Samples	Total Number of Positives	Percent Positive
Yes	50	9	18.0%
No	79	24	30.4%

Table 5. Seasonal distribution for black bear blood smears collected from Pennsylvania that were examined for *Dirofilaria* spp., 2018-2020. Seasons were categorized as the following: Spring was April-June, summer was July-September, fall was October-December, and winter was January-March. Season was not significantly associated with black bears being positive for microfilaria on blood smears.

Season	Total Number in Samples	Total Number of Positives	Percent Positive
Spring	61	14	30.0%
Summer	52	15	28.8%
Fall	8	4	50.0%
Winter	8	0	0.0%

Table 6. Summary of p-values showing significance of correlation between *Dirofilaria* spp. on blood smears and weight, mange presence, sex, and age variables calculated from binary logistic regression model excluding season variable and then including season variable without winter results.

Variable	P-value (excluding season variable)	P-value (season included without winter data)
Weight	0.146	0.210
Mange Presence	0.039	0.033
Season	-	0.844
Sex	0.705	0.949
Age	0.391	0.331

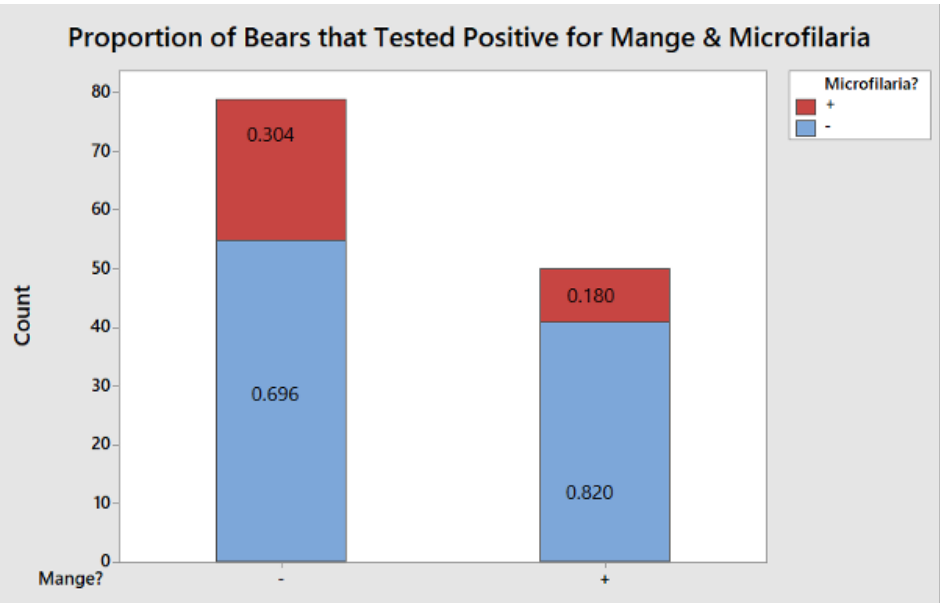

Figure 1. Proportional comparison of *Dirofilaria* spp. on blood smears and confirmed sarcoptic mange in black bears from Pennsylvania, 2018-2020. Seasonal data is excluded.

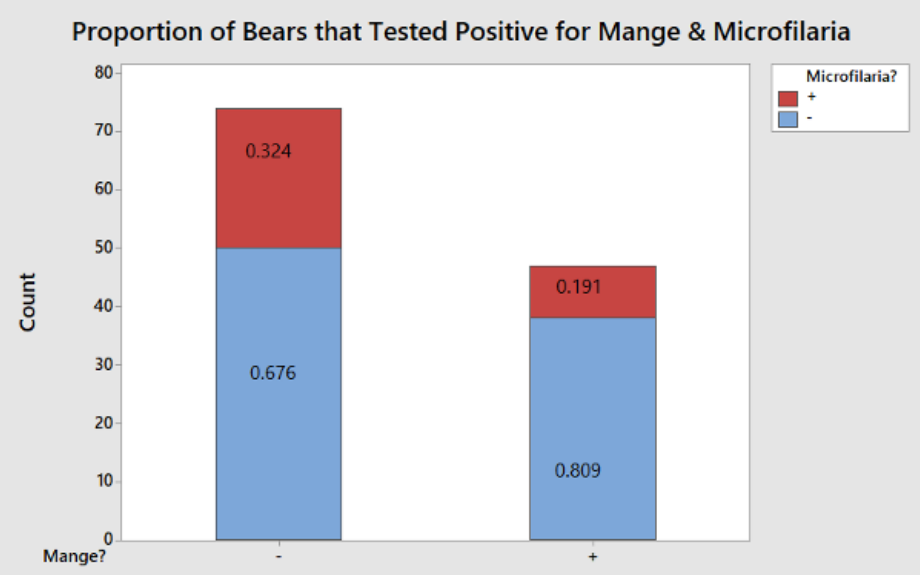

Figure 2. Proportional comparison of *Dirofilaria* spp. on blood smears and confirmed sarcoptic mange in black bears from Pennsylvania, 2018-2020. Seasonal data is included.

CHAPTER 4: DISCUSSION

The results of this study indicate that black bears in Pennsylvania are infected with *Dirofilaria* spp. at comparable levels to what has been reported in previous studies from the Upper Midwest, using similar diagnostic approaches. Overall, 25.6% (33/129) of the blood smears were positive for microfilaria that were morphologically consistent with *Dirofilaria* spp. This is similar to previous studies in Northern Wisconsin conducted in 1974-1975 and 2010, in which *Dirofilaria* spp. were identified on blood smears in 19% (17/90) and 21% (10/47), respectively. Although the differences in percent positives were minor between these studies, black bears in Pennsylvania during the study period experienced significant morbidity associated with sarcoptic mange, an emerging infectious disease in the Northeast. Sarcoptic mange, in this study, was found to have a statistically significant and inverse association with bears being positive for microfilaria (refer to paragraph below). Sarcoptic mange is a rare cause of morbidity or mortality in black bears in Wisconsin, both historically and currently. Consequently, the true difference in *Dirofilaria* spp. infection between Wisconsin and Pennsylvania black bears may be greater in the absence of sarcoptic mange. Based on morphology, none of the microfilaria were identified beyond *Dirofilaria* spp.; however, based on previous studies and the host species, we would assume these are *D. ursi*. Research is currently underway to confirm the species of these microfilaria through molecular techniques (i.e. PCR and sequencing).

Other than those with sarcoptic mange, none of the black bears that were positive for microfilaria exhibited any significant lesions or displayed any overt signs of disease prior to harvest, kill, or sampling. These results are consistent with the literature and support the concept

that *D. ursi* infection in black bears is asymptomatic. This is also consistent with existing literature on *D. immitis* in black bears, which has identified sporadic infections, but none that were associated with overt disease (Crum et al., 1978). Additional research is currently underway to further investigate the virulence of *Dirofilaria* spp. in black bears by evaluating CBC results in bears with confirmed microfilaria in the blood versus those in which the parasites were not detected.

This current study did not investigate associations between *Dirofilaria* spp. and *Wolbachia*, a symbiotic bacteria previously reported from *D. ursi*. The relationship between *Wolbachia* and *D. ursi* is poorly understood, but the impacts of *Wolbachia* on the pathogenesis of other filarial nematodes is more well defined. *Wolbachia* combined with *Wulchereria bancrofti* has been shown to elicit lymphatic filariasis (an inflammatory and immune response) and contribute to the resulting lymphatic disease (Roberts et al., 2009). *Wolbachia* has also been shown to contribute to dermatitis associated with onchocerciasis (a disease caused by *Onchocerca volvulus*); inflammation in this disease is worsened by the release of *Wolbachia* from dead juvenile filarial nematodes (Roberts et al., 2009). It currently is unknown whether *Wolbachia* plays a role in the virulence of *D. ursi* in bears; however, these data from other bacterial-parasitic symbiotic relationships suggest additional research is warranted.

Previous surveys for *D. ursi* in black bears have relied almost exclusively on hunter-harvested bears during the fall season. Consequently, none of these studies have evaluated the associations of demographic, seasonal, or environmental variables on *Dirofilaria* spp. infection in bears. In this study, sex, age, weight, and season did not have significant effects on *Dirofilaria* spp. infection. As is common with research on black bears, we experienced sampling bias that may have impacted our results. In this study we opportunistically collected samples when bears were

being handled for other projects or during hunting season. Consequently, the bulk of our samples come from summer (statewide tagging for population estimates), fall (hunting season), and winter (den visits on research bears), and most of the samples were from adult bears as opposed to juveniles. In addition, we sampled significantly more females than males due to the reliance on a long-term female reproduction study for bear handling/sampling. Interestingly, although season was not significantly associated with *Dirofilaria* spp. results, none of the bears (n=8) sampled in the winter, while in the den, were positive for microfilaria. This likely relates to the cold weather not being conducive to the biologic black fly vector. Future studies should try to collect samples more evenly distributed for these variables to confirm and expand upon these results.

One variable that was significantly associated with black bears being positive for microfilaria on blood smears was mange. Sarcoptic mange is a significant emerging infectious disease of black bears caused by the mite *Sarcoptes scabiei*. Sarcoptic mange was first identified in Pennsylvania in the early 1990s, and has since expanded throughout the Commonwealth and into adjacent states to the north and south (Niedringhaus et al., 2019). Black bears with confirmed sarcoptic mange, based on gross lesions and the presence of mites on skin scrapes, were significantly less likely to be positive for microfilaria on blood smears. The cause for this inverse relationship is unknown. However, two likely explanations are feasible, either alone or in concert. Adult *D. ursi* live in the subcutis (tissue below the skin) or in facia and soft tissue throughout body cavities. Bears that have sarcoptic mange often experience severe alopecia, crusting dermatitis, and secondary skin infections with bacteria and yeast. Skin lesions in bears can be severe and affect > 90% of their body. Consequently, the severe inflammation and damage to the cutaneous tissues may negatively impact the survival or reproduction of adult

nematodes in the subcutis, resulting in negative blood smear results. In addition, a significant component of the skin lesions and host response to sarcoptic mange is the result of a hypersensitivity reaction. Consequently, this systemic reaction may have negatively impacted the presence or survival of microfilaria in the blood. We are not aware of any reported associations between mange and *Dirofilaria* spp. infection in other host systems. Interestingly, existing data on *Dirofilaria* spp. infection in black bears in Pennsylvania indicate that this filarial nematode may be influenced by multiple other microorganisms, including positive associations (*Wolbachia*) and negative associations (*S. scabiei*). Additional research is needed to fully define these relationships, and what they may mean for the virulence and transmissibility of *Dirofilaria* spp.

Ongoing and Future Research

Efforts are currently underway to confirm the species of *Dirofilaria* spp. that were observed on blood smears from black bears, using molecular techniques (i.e. PCR and sequencing). In addition, bloodwork (i.e. CBC and blood chemistry) from black bears that are positive for *Dirofilaria* spp. are being analyzed for any abnormalities. Future studies are needed to further evaluate the epidemiology of *Dirofilaria* spp. in black bears. In addition, research is needed to better characterize the relationship between *Dirofilaria* spp., *Wolbachia*, and *S. scabiei*. Such information is necessary to better understand disease risks in black bears, as well as potential risks for humans.

Literature Cited

Anderson, R. C. (2000). *Nematode parasites of vertebrates: Their development and transmission* (2nd ed.). CABI Pub.

Arlian L.G., Runyan R.A., & Estes S.A. (1984). Cross infestivity of Sarcoptes scabiei. *Journal of the American Academy of Dermatology, 10*, 979–986. https://doi.org/10.1016/s0190-9622(84)80318-7

Artois, M., Bengis, R., Delahay, R. J., Duchêne, M .J., Duff, J. P., Ferroglio, E., Gortázar, C., Hutchings, M. R., Kock, R. A., Leighton, F. A., Mörner, T., & Smith, G. C. (2009). Wildlife disease surveillance and monitoring. In R. Delahay, G. C. Smith & M. R. Hutchings (Eds.), *Management of disease in wild mammals* (pp. 187-214). Springer, Tokyo, Japan.

Beaver, P. C., Wolfson, J. S., Waldron, M. A., Swartz, M. N., Evans, G. W., & Adler, J. (1987). Dirofilaria ursi-like parasites acquired by humans in the northern united states and canada: Report of two cases and brief review. *The American Journal of Tropical Medicine and Hygiene*, *37*(2), 357-362. https://doi.org/10.4269/ajtmh.1987.37.357

Bourne, D. C., Cracknell, J. M., & Bacon, H. J. (2010). Veterinary issues related to bears (Ursidae). *International Zoo Yearbook, 44*(1), 16-32. https://doi.org/10.1111/j.1748-1090.2009.00097.x

Bowman, D. D., & Atkins, C. E. (2009). Heartworm biology, treatment, and control. *The Veterinary Clinics of North America. Small Animal Practice, 39*(6), 1127-1158. https://doi.org/10.1016/j.cvsm.2009.06.003

Canestri Trotti, G., Pampiglione, S., & Rivasi, F. (1997). The species of the genus Dirofilaria, Railliet & Henry, 1911. *Parassitologia, 39*(4), 369.

Catalano, S., Lejeune, M., Tizzani, P., Verocai, G. G., Schwantje, H., Nelson, C., & Duignan, P. J. (2015). Helminths of grizzly bears (Ursus arctos) and American black bears (Ursus americanus) in Alberta and British Columbia, canada. *Canadian Journal of Zoology, 93*(10), 765-772. https://doi.org/10.1139/cjz-2015-0063

Crum, J., Nettles, V., & Davidson, W. (1978). Studies on endoparasites of the black bear (Ursus americanus) in the southeastern United States. *Journal of Wildlife Diseases, 14*(2), 178-186. https://doi.org/10.7589/0090-3558-14.2.178

Decker, D., & Purdy, K. (1988). Toward A Concept of Wildlife Acceptance Capacity in Wildlife Management. *Wildlife Society Bulletin (1973-2006), 16*(1), 53-57.

Desch C.E., Jr. (2009). A new species of *Demodex* (Acari: Demodecidae) from the black bear of North America, *Ursus americanus* pallas, 1780 (Ursidae) *International Journal of Acarology, 21*:23–26. https://doi.org/10.1080/01647959508684039

Diefenbach, D. R., & Alt, G. L. (1998). Modeling and evaluation of ear tag loss in black bears. *The Journal of Wildlife Management, 62*(4), 1292-1300. https://doi.org/10.2307/3801993

Di Salvo, A. R., & Chomel, B. B. (2020). Zoonoses and potential zoonoses of bears. *Zoonoses and Public Health, 67*(1), 3-13. https://doi.org/10.1111/zph.12674

Duffy, M. S., Greaves, T. A., & M. D. B. Burt. (1994). Helminths of the black bear, Ursus americanus, in New Brunswick. *The Journal of Parasitology, 80*(3), 478-480. https://doi.org/10.2307/3283422

Ellen, R. F. (1982). *Environment, subsistence, and system: The ecology of small-scale social formations*. Cambridge University Press.

Foster, G., Cames, T., & Forrester, D. (1998). Geographical distribution of Demodex ursi in black bears from Florida. *Journal of Wildlife Diseases, 34*(1), 161-164. https://doi.org/10.7589/0090-3558-34.1.161

Frechette, J. -L., & Rau, M. E. (1977). Helminths of the black bear in Quebec. *Journal of Wildlife Diseases, 13*(4), 432-434. https://doi.org/10.7589/0090-3558-13.4.432

Garcia, L. S. (2009). Classification and Nomenclature of Human Parasites. In *Textbook of Pediatric Infectious Diseases* (pp. 286 -2865).

Gwynn, A. D., Sharkey, L., Gerhold, R., Holland, A., Isaac, E. J., Michalski, S., & Teigen, L. (2017). What is your diagnosis? Blood film from a black bear (Ursus americanus). *Veterinary Clinical Pathology, 46*(4), 643-644. https://doi.org/10.1111/vcp.12531

Haldane, D. J., Johnston, B. L., & Walsh, N. M. (1996). Subcutaneous dirofilariasis in Nova Scotia. *Canadian Journal of Infectious Diseases*, *7*(1), 67-69. https://doi.org/10.1155/1996/378616

Herzberg, A. J., Boyd, P. R., & Gutierrez, Y. (1995). Subcutaneous dirofilariasis in Collier County, Florida, U.S.A. *The American Journal of Surgical Pathology, 19(*8), 934-939. https://doi.org/10.1097/00000478-199508000-00009

Liu, R. Z., & Borthwick, A. G. L. (2011). Measurement and assessment of carrying capacity of the environment in Ningbo, China. *Journal of Environmental Management, 92*(8), 2047-2053. https://doi.org/10.1016/j.jenvman.2011.03.033

MacDonald, A. M., Johnson, J. B., Casalena, M. J., Nemeth, N. M., Kunkel, M., Blake, M., & Brown, J. D. (2020). Active and Passive Disease Surveillance in Wild Turkeys (Meleagris gallopavo) from 2008-2018 in Pennsylvania, USA.

Manville AM, , 2nd. (1978). Ecto- and endoparasites of the black bear in northern Wisconsin. *Journal of Wildlife Diseases, 14*(1), 97-101. https://doi.org/10.7589/0090-3558-14.1.97

Merriam-Webster. (2021). Disease. In *Merriam-Webster.com Dictionary*. Retrieved from https://www.merriam-webster.com/dictionary/disease#medicalDictionary.

Merriam-Webster. (2021). Etiology. In *Merriam-Webster.com Dictionary*. Retrieved from https://www.merriam-webster.com/dictionary/etiology#medicalDictionary.

Merriam-Webster. (2021). Pathogen. In *Merriam-Webster.com Dictionary*. Retrieved from https://www.merriam-webster.com/dictionary/pathogen#medicalDictionary.

Merriam-Webster. (2021). Poach. In *Merriam-Webster.com dictionary*. Retrieved from https://www.merriam-webster.com/dictionary/poach.

Michalski, M. L., Bain, O., Fischer, K., Fischer, P. U., Kumar, S., & Foster, J. M. (2010). Identification and phylogenetic analysis of Dirofilaria ursi (Nematoda: Filarioidea) from Wisconsin black bears (Ursus americanus) and its Wolbachia endosymbiont. *Journal of Parasitology, 96*(2), 412-419. https://doi.org/10.1645/GE-2208.1

Mortenson, J. A. (1998, November 18). *Serologic Survey of Infectious Disease Agents in Black Bears (Ursus americanus) of California, Oregon, and Washington* [Master's Thesis, Oregon State University].

Niedringhaus, K. D., Brown, J. D., Sweeley, K. M., & Yabsley, M. J. (2019). A review of sarcoptic mange in North American wildlife. International Journal for Parasitology. *Parasites and Wildlife, 9*, 285-297. https://doi.org/10.1016/j.ijppaw.2019.06.003

Pence, D.B. & Ueckermann, E. (2002). Sarcoptic mange in wildlife. *Rev. Sci. Tech., 21*:385–398.

Pennsylvania Game Commission. (2021). *Black Bear Population Growth*. https://www.pgc.pa.gov/Wildlife/WildlifeSpecies/BlackBear/Pages/BlackBearPopulationGrowth.aspx.

Pennsylvania Game Commission. (2021). *Black Bear Wildlife Note*. https://www.pgc.pa.gov/Wildlife/WildlifeSpecies/BlackBear/Pages/default.aspx

Pennsylvania Game Commission. (2021). *Big Game Harvest Dat*a. https://www.pgc.pa.gov/HuntTrap/Hunting/HarvestDataandMaps/Pages/Big%20Game%20Harvest%20Data.aspx

Pennsylvania Game Commission. (2021). *Mange in Black Bears*. https://www.pgc.pa.gov/Wildlife/WildlifeSpecies/BlackBear/Documents/Mange%20in%20Black%20Bears.pdf

Poppert, S., Hodapp, M., Krueger, A., Hegasy, G., Niesen, W. D., Kern, W. V., & Tannich, E. (2009). Dirofilaria repens infection and concomitant meningoencephalitis. *Emerging Infectious Diseases, 15*(11), 1844-1846. https://doi.org/10.3201/eid1511.090936

Responsive Management. (2008). Pennsylvania Residents' Opinions on and Attitudes toward Black Bears.

Roberts, L. S., Schmidt, G. D., & Janovy, J., Jr. (2009). *Gerald D. Schmidt & Larry S. Roberts' Foundations of Parasitology* (8th ed.). McGraw-Hill Higher Education.

Rogers, L. (1975). Parasites of black bears of the Lake Superior region. *Journal of Wildlife Diseases, 11*(2), 189-192. https://doi.org/10.7589/0090-3558-11.2.189

Rogers, L. L. (1977). *Social relationships, movements, and population dynamics of black bears in northeastern Minnesota* [Ph.D. Dissertation, University of Minnesota].

Rogers, L. L., & Rogers, S. M. Parasites of Bears: A Review. *Third International Conference on Bears.*

Saari, S., DVM, Näreaho, A., & Nikander, S. (2019;2018;). *Canine parasites and parasitic diseases*. Academic Press, an imprint of Elsevier.

Samuel, W. M., Kocan, A. A., Pybus, M. J., & Davis, J. W. (2001;2000;). *Parasitic diseases of wild mammals* (2nd ed.). Iowa State University Press.

Seidl, I., & Tisdell, C. A. (1999). Carrying capacity reconsidered: From Malthus' population theory to cultural carrying capacity. *Ecological Economics, 31*(3), 395-408. https://doi.org/10.1016/S0921-8009(99)00063-4

Sommerer, A.S. (2014). *A Spatial Analysis of the Relationship between the Occurrence of Mange in Pennsylvania's Black Bear Population and Impervious Land Cover* [Master's Thesis, Indiana University of Pennsylvania].

Stallknecht, D. E. (2007). *Impediments to wildlife disease surveillance, research, and diagnostics.* (pp. 445-461). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-70962-6_17

Stephenson, N., Higley, J. M., Sajecki, J. L., Chomel, B. B., Brown, R. N., & Foley, J. E. (2015). Demographic characteristics and infectious diseases of a population of American black bears in Humboldt County, California. *Vector Borne and Zoonotic Diseases (Larchmont, N.Y.), 15*(2), 116.

Ternent, M. (2006). *Management and Biology of Black Bears in Pennsylvania: Ten Year Plan* (2006-2015).

Ternent, M. (2019). Black Bear Research and Management. *Pennsylvania Game Commission Bureau of Wildlife Management Project Annual Job Report*.

Uni, S. (1983). Filarial parasites from the black bear of Japan. A*nnales De Parasitologie Humaine Et Comparée, 58*(1), 71-84. https://doi.org/10.1051/parasite/1983581071

Vincent, A. L., Greene, J., Tucci, V., & Cabrera-Cancio, M. R. (2013). Dirofilaria tenuis causing neuritis. *Infectious Diseases in Clinical Practice (Baltimore, Md.), 21*(5), 325-329. https://doi.org/10.1097/IPC.0b013e318279f192

Wimsatt, W. A. (1963). Delayed implantation in the Ursidae, with particular reference to the black bear (Ursus americanus Pallus). In A. C. Enders (Eds.), *Delayed implantation* (pp. 49-76). University of Chicago Press, Chicago, Illinois.

Wyatt, D., Santoro, D., Deabold, K., Gruntmeir, J., Childress, A., Craft, W. F., Walden, H. D. S., & Wellehan, J. F. X. (2020). Subcutaneous nodules and dermatitis associated with non-immitis non-repens dirofilariosis morphologically consistent with Dirofilaria striata in a 2-year-old male domestic cat in Florida, USA. *The Veterinary Quarterly, 40*(1), 215-222. https://doi.org/10.1080/01652176.2020.1814972

Yamada, M., Shishito, N., Nozawa, Y., Uni, S., Nishioka, K., & Nakaya, T. (2017). A combined human case of Dirofilaria ursi infection in dorsal subcutaneous tissue and anisakis simplex sensu stricto (s.s.) infection in ventral subcutaneous tissue. *Tropical Medicine and Health, 45*(1), 26-6. https://doi.org/10.1186/s41182-017-0067-4

Yokohata, Y., Fujita, O., Kamiya, M., Fujita, T., Kaneko, K., & Ohbayashi, M. (1990). Parasites from the Asiatic black bear (Ursus thibetanus) on Kyushu Island, Japan. *Journal of Wildlife Diseases, 26*(1), 137-138. https://doi.org/10.7589/0090-3558-26.1.137

Yunker, C., Binninger, C., Keirans, J., Beecham, J., & Schlegel, M. (1980). Clinical mange of the black bear, Ursus americanus, associated with Ursicoptes americanus (acari: Audycoptidae). *Journal of Wildlife Diseases, 16*(3), 347-356. https://doi.org/10.7589/0090-3558-16.3.347

ACADEMIC VITA

Alia Mazzatosta
arm6122@psu.edu

EDUCATION

The Pennsylvania State University, Spring 2021

Bachelor of Science Degree in Veterinary and Biomedical Sciences

Schreyer Honors College

Honors in Veterinary and Biomedical Sciences

Thesis Title: Surveillance for *Dirofilaria* spp. in American Black Bears (*Ursus americanus*) in Pennsylvania, 2018-2020

Thesis Supervisor: Dr. Justin Brown

RELATED EXPERIENCE:

Harveys Lake Veterinary Clinic (Summer 2019)

The Rabbit Resource Foster Program Volunteer (Summer 2019)

Electric City Aquarium Volunteer (Summer 2020)

AWARDS:

Gamma Sigma Delta, the Honor Society of Agriculture

Dean's List

Top 1% Honor Roll College of Agricultural Sciences

Rosemarie C. and Howard R. Peiffer Scholarship in the College of Agricultural Sciences

Rosie and Stuart Kahan Scholarship in Pre-Veterinary Sciences

Gerald and Leoda Gummo Excellence Fund in Agriculture

Irene Thomas Memorial Award

ACTIVITES:

Penn State Wilkes-Barre Women's Volleyball

Vice President of Penn State Wilkes-Barre's Blue and White Society

Acting Vice Present of Penn State Wilkes-Barre's Four Seasons Club

Penn State Wilkes-Barre Lion Ambassador

Penn State Wilkes-Barre THON Club

Penn State University Park Pre-Vet Club