Tapeworms of dogs and cats in North America

by

Kamilyah R. Miller

B.S., Florida Agricultural and Mechanical University, 2013
D.V.M., Tuskegee University, 2018

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine & Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2025

Abstract

Dipylidium caninum and *Echinococcus multilocularis* are zoonotic tapeworms that infect domestic dogs and cats worldwide. *Dipylidium caninum* is transmitted by *Ctenocephalides felis* (the cat flea); recent reports show two host-adapted *D. caninum* strains, cat and dog, and resistance to praziquantel. *Echinococcus multilocularis,* transmitted by rodents, causes alveolar echinococcosis (AE) in domestic dogs and humans, and is a major public health concern in North America. This thesis addresses gaps in the prevalence of *D. caninum* in fleas and *E. multilocularis* in wild canids and the need to improve molecular diagnostics for *E. multilocularis* in domestic dogs and wild canids. In Chapter 2, our lab utilized a useful method for pool testing fleas for surveillance and assessing infection rate of *D. caninum*. This work demonstrated the prevalence of *D. caninum* in fleas of 3.8% through pool testing of fleas collected from the environment and on-animal. Molecular epidemiology also revealed the feline genotype of *D. caninum* was present in fleas collected from cats, matching *D. caninum* cat strains previously reported. In Chapter 3, our surveillance revealed that 47% of coyotes in Kansas and 42% in Missouri are infected with *Echinococcus multilocularis*, a new endemic region, plus a single red fox in Missouri. Additionally, 20% of coyotes in Illinois and 16% in Indiana were infected with *E. multilocularis*, which are known endemic regions. Molecular epidemiology revealed the European haplotype is present in wild canids in the Midwest United States, instead of the, previously reported, N2 North American haplotype. Thus, documents the expanding range of *E. multilocularis* and the highly pathogenic and zoonotic Europeans strains in North America. In Chapter 4, our work concluded that adult cestode recovery remains 'the gold standard' when compared to common diagnostic techniques such as fecal flotation, sedimentation and copro-PCR. I described the importance of developing a reliable method for extracting taeniid egg DNA

for large scale surveillance of *E. multilocularis* in domestic dogs and wild canids. This thesis provides useful methods for surveillance of *D. caninum* in fleas through pool testing and data on the prevalence of *Echinococcus multilocularis* that can aid in the design of control and prevention programs.

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Approved by:

Major Professor
Brian H. Herrin DVM, PhD, DACVM
(Parasitology)

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Dedication

To God, my Lord and Savior, because without him none of this is possible.

To my grandparents, Katherine S. Ross, Blanche & Benjamin Miller, who are cheering me on in heaven!

List of Abbreviations

AE:	Alveolar Echinococcosis
Bp:	Base Pair
CDC:	Center for Disease Control
CE:	Cystic Echinococcosis
COI:	Cytochrome C oxidase subunit I
CT:	Computed tomography
dH_2O:	Distilled H_2O
DNA:	Deoxyribonucleic Acid
ELISA:	Enzyme-Linked Immuno-Sorbent Assay
gDNA:	Genomic Deoxyribonucleic Acid
IH:	Intermediate Host
MRI:	Magnetic resonance imaging
NCR:	North Central Region
NTZ:	Northern Tundra Zone
PCR:	Polymerase Chain Reaction
PZQ:	Praziquantel
qPCR	Quantitative Polymerase Chain Reaction
SCT:	Sedimentation and Counting Technique
SFCT:	Sedimentation, Filtration, and Counting Technique
SPG:	Specific Gravity
WHO:	World Health Organization

Chapter 1 - General Introduction to Tapeworms of Veterinary Importance

1.1 General Introduction to Tapeworms

Tapeworms are flat, segmented, hermaphroditic worms that infect a variety of aquatic and terrestrial animals as definitive and intermediate hosts (Conboy, 2009). The two orders of veterinary importance are Diphyllobothriidea and Cyclophyllidea (Bowman, 2021).

In general, all adult tapeworms have a head with a holdfast organ on the anterior end, followed by a short, unsegmented section (neck) before segmentation begins (body) (Soulsby, 1968). The holdfast organ may be primitive with two bilateral slit-like grooves called bothria (seen in Diphyllobothriidea) or more complex with a rostellum and suckers (Bowman, 2021; Waeschenbach et al., 2017). The rostellum is located on the anterior end of the scolex. The rostellum is a conelike structure that can be retracted into the head of the tapeworm, or non-retractable. The rostellum can also be armed with hooks or unarmed without hooks. The segmented tapeworm body, or strobila, consists of individual reproductive segments called proglottids that mature along the length of the strobila. Proglottids can vary in shape and size, have one or two sets of reproductive organs, and be gravid (contains eggs) or senile (contain no eggs). Adult tapeworms lack true mouthparts and a digestive tract; therefore, all nutrients are absorbed through the tegument (Conboy, 2009).

Tapeworms can infect definitive hosts through a variety of aquatic or terrestrial indirect life cycles that have co-evolved with the definitive hosts feeding and grooming behaviors. All tapeworms that infect domestic dogs and cats require one or two intermediate hosts before becoming infectious to the dog or cat (Conboy, 2009). The lifecycle of tapeworms begins when eggs or gravid proglottids are passed in the feces of infected definitive hosts. Eggs are released into the environment and incidentally ingested by the intermediate host. The metacestode, or larval stage, develops within the intermediate host, which then becomes infectious to the definitive host, with some lifecycles requiring a second intermediate host to reach the infective stage (Kuchta et al., 2024; McAllister et al., 2018; Oehm et al., 2024). The adult tapeworms subsequently mature in the small intestine, completing the life cycle.

Clinical disease is typically mild or not seen in adult tapeworm infections of domestic dogs and cats (Evason et al., 2025; Kuroki et al., 2020). In fact, in many cases, such as with *Spirometra* spp. and *Echinococcus* spp., it is the metacestode stages that cause more clinical disease than adult infections (Drake et al., 2008; Kuroki et al., 2022; Williams & Walzthoni, 2023). Diagnosis and treatment vary depending on the affected host, clinical signs, and tapeworm species and stage. The standard for tapeworm egg recovery and identification is by centrifugal fecal flotations using Sheather's sugar (specific gravity 1.27) or fecal sedimentations in water (A. M. Zajac et al., 2021). Commonly, tapeworm infections are discovered by the owner when fresh proglottids are found in the feces, perianally, or dried and adhered to the fur on the hindlegs of infected animals. These fresh and/or dried proglottids can be hydrated in saline, squashed onto a microscope slide to release eggs, and then the eggs can be used to identify the tapeworm species (A. M. Zajac et al., 2021).

Adult tapeworm infections of small animals are most commonly treated using praziquantel, given in different formulations and dosages that vary depending on the host, tapeworm species, and stage being treated (Bowman, 2021; Riviere & Papich, 2018). In order to prevent infection of the metacestode stages, domestic dogs and cats should be discouraged from coprophagy of feces potentially infected with infectious eggs and ingestion of potentially infected intermediate hosts (Drake et al., 2008; Evason et al., 2025; Kuroki et al., 2022). To prevent infection with adult stages, dogs and cats should be kept away from intermediate hosts, which may be difficult given the range of vertebrate and invertebrate hosts (Kuroki et al., 2020; McAllister et al., 2018; McAllister & Conn, 1990). For year-round protection, domestic dogs and cats can be placed on a monthly broad-spectrum product, although the commercially available options are only effective on adult stages of Cyclophyllidean tapeworms (Bowman, 2021).

1.2 Tapeworms of Veterinary Importance

1.2.1 Order Diphyllobothriidae

Diphyllobothriidean tapeworms, formerly known as Pseudophyllidean tapeworms, are broad tapeworms of wild animals that infect a wide range of hosts including frogs (Family Cephalochlamydidae), reptiles (Family Scyphocephalidae), birds and mammals (Family Diphyllobothriidae) (Kuchta et al., 2008, 2024). The tapeworm species of most veterinary and medical importance are found in the Diphyllobothriidean family. The most common Diphyllobothriidean tapeworms found in domestic dogs and cats and humans are from the genera *Dibothriocephalus*, found mainly in cold weather climates, and *Spirometra*, found mainly in warm weather climates (Scholz et al., 2019).

1.2.1.1 Family Diphyllobothriidae

1.2.1.1.1 *Dibothriocephalus latus*

Taxonomy

There have been many suggestions on the correct taxonomic classification of broad tapeworms that infect terrestrial hosts (Kuchta et al., 2024). *Dibothriocephalus* was once an established genus by Lühe (1899), but ten years later the same author combined this genus with *Diphyllobothrium*, an established genus by Cobbold (1858) (Waeschenbach et al., 2017). This regrouping was widely accepted with a few proposed taxonomic changes throughout the years, including recent suggestions of splitting the *Diphyllobothrium* genus into three subgenera: *Dibothriocephalus* spp. for parasites of terrestrial mammals and birds, *Diphyllobothrium* sensu stricto for parasites of cetaceans, and an unnamed *Diphyllobothrium* group of parasites from pinnipeds (Kuchta et al., 2024). However, morphological features and molecular testing support the idea that *Diphyllobothrium* is a polyphyletic genus and not monophyletic. Thus, *Diphyllobothrium* has been reserved for parasites that infect cetaceans, or marine mammals, and the genus *Dibothriocephalus* has been resurrected to include terrestrial and freshwater species, including *Dibothriocephalus latus* that infect domestic dogs and cats and humans (Waeschenbach et al., 2017).

Morphology

<u>Egg</u>

Eggs are light brown, subspherical, operculate, unembryonated with a smooth surface when shed in the feces (Waeschenbach et al., 2017). The eggs are relatively large, measuring 66 x 44 µm, and are golden brown.

<u>Metacestode</u>

The oncosphere, tapeworm larva, develops inside a ciliated embryopore called a coracidium. The procercoid, first metacestode stage, is a solid body with a cercomer, a caudal appendix with hooks, with an unknown function (Hammerschmidt & Kurtz, 2007). The cercomer is shed with the outer lining when the procercoid develops into the plerocercoid (Hammerschmidt & Kurtz, 2007). The plerocercoid, second metacestode stage, has an elongated, solid body with adult mouthparts (Soulsby, 1982).

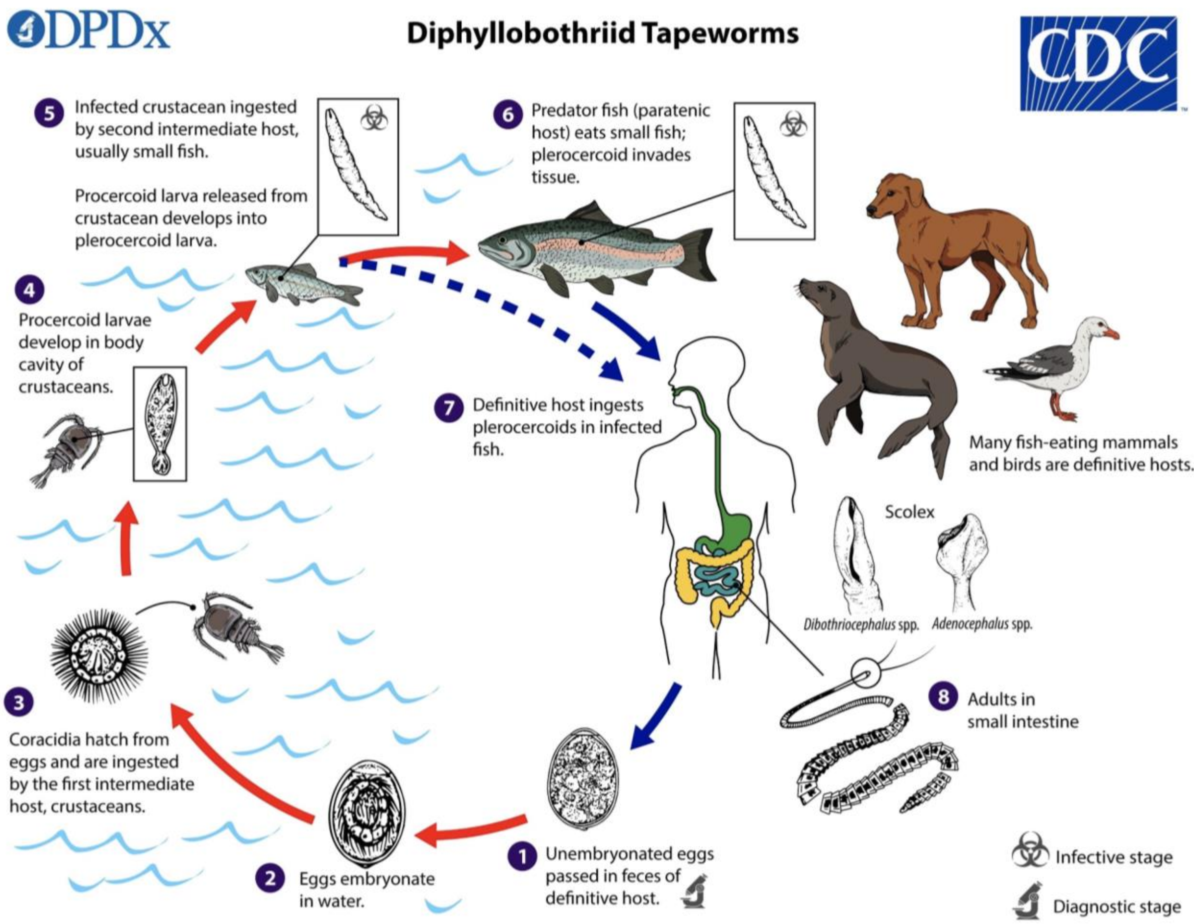
<u>Adult</u>

Adults of *D. latus*, the ‘broad fish tapeworm’, can reach 1 meter, or longer, in length. The head has an elongated, spoon-shaped, slit-like bothria, or the holdfast organ, that open anteriorly and posteriorly. The neck is long and narrow with the strobila containing proglottids that are wider than they are long. Sexually mature proglottids have one set of reproductive organs. The ovaries are bilobed and rosette shaped located medially in the proglottid. The testes are numerous and located in the cortex of the proglottid. The uterine pore is located on the medioventral surface of the proglottid (Waeschenbach et al., 2017).

Life Cycle

The life cycle, as illustrated in Figure 1.1, begins when operculated, unembryonated eggs are shed in the feces of infected animals. In water, the coracidium develops inside the egg, hatches and then is ingested by the first intermediate host, a *Diaptomus* spp. copepod. Inside the copepod, the oncosphere develops into a procercoid (Hatsushika et al., 1981; Scholz et al., 2019). The infected copepod is then ingested by the 2nd intermediate host, a freshwater or brackish water fish, and the procercoid develops into a plerocercoid in the fish musculature (Kuchta et al., 2015; Kuchta & Scholz, 2017)(Dubinina, 1980). The plerocercoid can survive predation of the infected 2nd intermediate host and migrate to the musculature of a paratenic host. The infected 2nd intermediate host, or paratenic host, is ingested by the definitive host, a domestic dog or cat. The plerocercoid uses the adult bothria and attaches to the intestinal wall to mature into an adult and segment.

Figure 1.1 Life cycle of *Dibothriocephalus latus*. Definitive hosts include fish eating mammals such as domestic canids, seals and birds, while intermediate hosts include *Diaptomus* spp. copepods first and small fish second (CDC, 2019a).



Prevalence

Diphyllobothrium tapeworms have a cosmopolitan distribution, with most species occurring in cold weather climates (Scholz & Kuchta, 2016). *Dibothriocephalus latus* has been most commonly reported in North America, in the Canadian provinces of Manitoba, Saskatchewan, Alberta, and the forested lakes of Ontario, and around Lake Superior in the United States (Scholz & Kuchta, 2016). *Dibothriocephalus latus* adults have been found in humans, domestic dogs and cats, wild canids and felids, and raccoons (Scholz & Kuchta, 2016). The prevalence of *D. latus* in domestic dogs and cats ranges from 2 to 50% (Scholz et al., 2019).

Metacestode stages have been found in freshwater fish, most commonly found in pike, perch, and burbot and less commonly in ruff, pikeperch and yellow perch (Scholz & Kuchta, 2016).

Clinical Disease

Typically, infected domestic dogs have subclinical gastrointestinal signs. However, experimental infections showed decreased red blood cell counts and hemoglobin levels (Wardle et al., 1947).

Zoonosis

Diphyllobothriosis infects an estimated 20 million people worldwide, with six species considered true human parasites: *Andenocephalus pacificus*, *Diphyllobothrium balaenopterae*, *Diph. stemmacephalum*, *Dibothriocephalus dendriticus*, *Diboth. latus*, and *Diboth. nihonkainensis* (Muller et al., 2002; Waeschenbach et al., 2017). *Dibothriocephalus latus* and *Diboth. nihonkaiensis*, are considered true parasites of humans because the adult tapeworms grow at a faster rate in humans than domestic dogs, cats, wolves and foxes (Von Bonsdorff, 1977). Most human diphyllobothriosis cases have been reported from Japan, but autochthonous infections have also been reported from the Alpine lakes (Lakes Maggiore, Como, Iseo, and Garda), Baltic countries and Russia in Europe; China, Korea, and Russia in Asia; Peru in South America; and Canada and the Great Lakes and Pacific coast in northern United States in North America (Gustinelli et al., 2016; Kuchta et al., 2015; Scholz & Kuchta, 2016). In the United States, cases from California and North Carolina were molecularly confirmed as *D. nihonkaiensis* after initially being diagnosed as *D. latus* (Scholz & Kuchta, 2016). An increase in human infections worldwide is attributed to a few factors: i) increased demand for fish, ii) increased popularity for consumption of raw or undercooked fish in common cuisines such as ceviche, sushi, and sashimi, fish carpaccio, and iii) importation of chilled or insufficiently frozen fish, international travel, and migration of people (Arizono et al., 2009; Broglia & Kapel, 2011;

Kuchta et al., 2015; Waeschenbach et al., 2017). Diphyllobothriosis causes clinical disease in humans exhibited as mild abdominal discomfort, nausea, diarrhea, constipation, and weakness. Adult *Diboth. latus* absorb Vitamin B_{12} from the gastrointestinal tract and, as a result, megaloblastic anemia can develop in heavy infections (Hochberg & Bhadelia, 2015). Diphyllobothriosis in humans has been successfully treated with praziquantel and niclosamide (Scholz et al., 2019).

1.2.1.1.2 Spirometra spp.

Taxonomy

Spirometra is a taxonomically complicated group of tapeworms with major challenges in the systematics and species identification (Kuchta & Scholz, 2017). *Spirometra* was once thought to be synonymous with *Diphyllobothrium*, but molecular data confirmed *Spirometra* as a separate, valid genus (Waeschenbach et al., 2017). The variability of adult Spirometra morphology between tapeworm specimens found in the same host and even proglottids on the same strobila has made it difficult to definitively identify *Spirometra* spp. based off morphology alone (Kuchta et al., 2024). Therefore, molecular techniques targeting the cytochrome c oxidase subunit I (COI) gene from preserved samples have been used to determine *Spirometra* taxonomy (Kuchta et al., 2024).

Currently, there are seven accepted *Spirometra* species: *Spirometra mansoni* found worldwide, *S. asiana* in Japan in Korea, *S. theileri* in Africa, *S. erinaceiuropaei* in Europe, *S. decipiens* in South America, *Spirometra* sp. 2 (American lineage) in North and South America, and *Spirometra* sp. 3 (North American lineage) found in the United States, which was historically reported as *Spirometra mansonoides* (Kuchta et al., 2024). Recently, morphological and molecular evidence indicate that *S. mansonoides* could potentially be a separate species, but more data is needed for a final determination (Kuchta et al., 2024). Therefore, all *Spirometra* isolates from the United States are currently referred to as *Spirometra* sp. 3 (Kuchta et al., 2024).

Morphology

Egg

Eggs are light brown, subspherical, operculate, unembryonated with a smooth surface (Waeschenbach et al., 2017) when shed in the feces. They are relatively large, measuring 66 x 44 µm, and a golden brown.

Metacestode

The oncosphere, the tapeworm embryo, develops inside a ciliated embryopore, and measures around 43.8 x 36.9 µm. The first metacestode stage is a procercoid, a solid body with a cercomer, a caudal appendage with embryonic hooks and an unknown function. The second metacestode stage is the plerocercoid, also referred to as sparganum, an elongated, solid body with adult mouthparts.

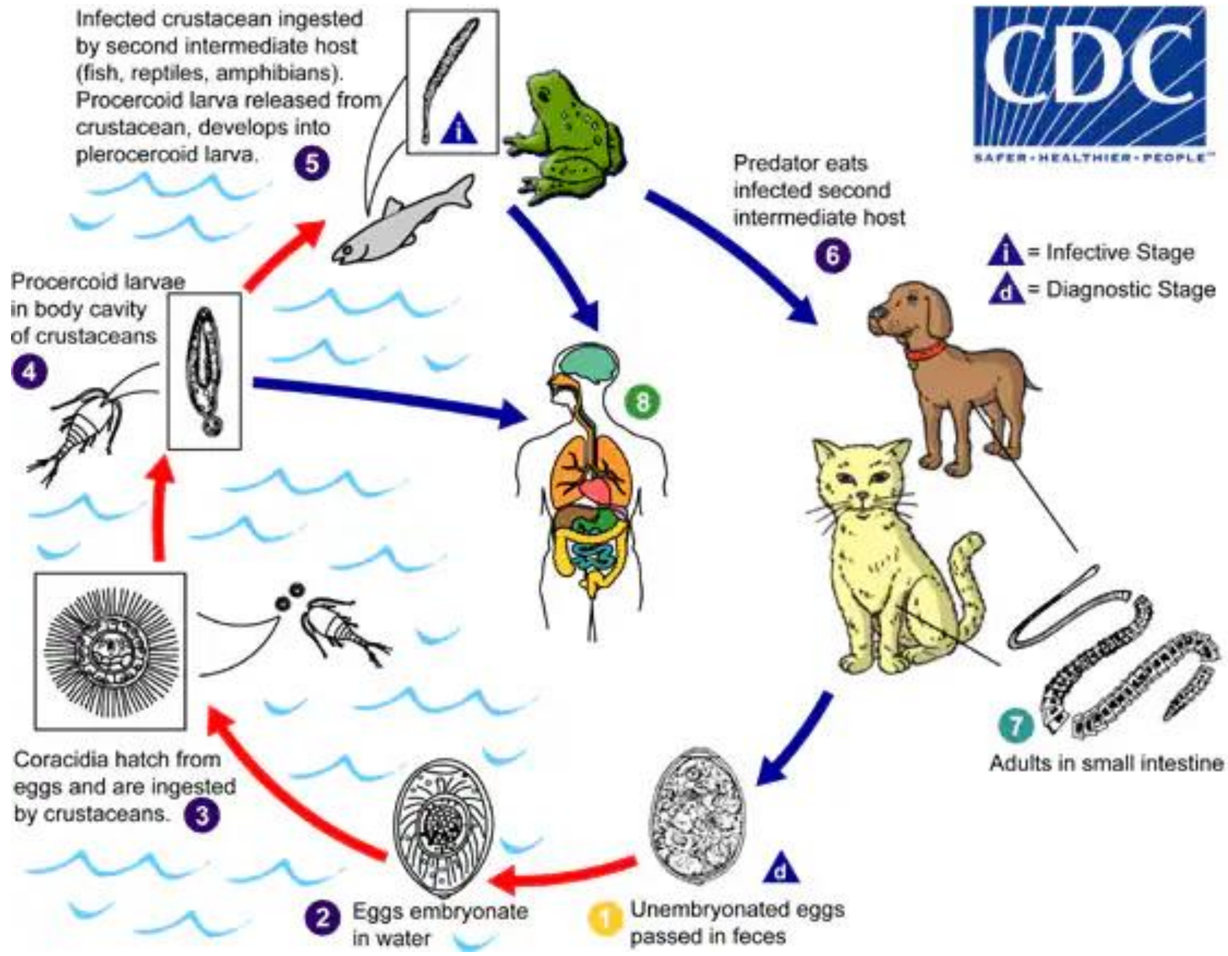
Adult

Adult *Spirometra* spp. can reach 1.5 meters in length and use bothria as the holdfast organ located on the anterior end of the tapeworm. The bothria are elongated, slit-like, and open both anteriorly and posteriorly. The neck of *Spirometra* spp. is long and narrow followed by immature, mature, and gravid proglottids that make up the tapeworm body. Proglottids are wider, than they are long, and the uterine pore is located on the medioventral surface. Mature proglottids have one set of reproductive organs and many testes located on both sides of the uterus. Adults of *Spirometra* sp. 3 (reported as *S. mansonoides*) have a spiraled uterus and *S. decipiens* has a rosette shaped uterus in the middle of the proglottid (Kuchta et al., 2024).

Life Cycle

Spirometra spp. commonly infect domestic cats more frequently than domestic dogs and a variety of wild carnivore hosts, mainly bobcats, as definitive hosts (Kuchta et al., 2024; Verocai et al., 2023). The lifecycle, illustrated in Figure 1.2, begins when adults shed operculated eggs in the feces of infected animals. A coracidium hatches out the egg when it touches water and is ingested by a *Cyclops* spp. copepod, the 1st intermediate host. Within the body cavity of the copepod, a procercoid develops (Kuchta et al., 2024). The infected copepod is then ingested by a fish, amphibian, or reptile, 2nd intermediate host, and a plerocercoid, or sparganum, the infective stage, develops in the connective tissue, or musculature, of the infected intermediate host. The plerocercoid, or sparganum, can survive predation of the infected 2nd intermediate host and migrate to the musculature of the paratenic host (Kuchta et al., 2024). When the infected 2nd intermediate or paratenic host is ingested by the domestic cat or dog, the adult scolex attaches to the intestinal wall and the tapeworm begins to segment and mature.

Figure 1.2 Life cycle of *Spirometra* spp. Felid and canid definitive hosts include domestic cats, bobcats, and domestic dogs, while intermediate hosts include *Cyclops* spp. copepods first and fish, reptiles and amphibians second **(CDC, 2017)**.



Prevalence

In North and South America, four *Spirometra* spp. have been reported: *Spirometra mansoni*, *Spirometra decipiens* and *Spriometra* sp. 2 and sp. 3. In South America, *S. decipiens* is found in wild felids, and *Spirometra* sp. 2 is found in a variety of wild felids and domestic cats and dogs, with one isolate found in a bobcat from Illinois, USA (Kuchta et al., 2021). In the United States, *Spirometra* sp. 3 (includes samples reported as *S. mansonoides*) is the main species found in wild and domestic felids (Kuchta et al., 2021). Recently, *S. mansoni* was reported in a crab-eating fox in Columbia in South America and a captive samar cobra (*Naja samarensis*) in the southern United States (Brabec et al., 2022; Verocai et al., 2023).

Spirometra spp. tapeworms have been reported in 24 states in the United States. *Spirometra* spp. adults have been found more frequently in domestic cats than domestic dogs from the Southeastern and Gulf Coast states, as well as Hawaii, New Jersey, New York and Pennsylvania. Surveillance studies show 2.9% (3/103) of shelter cats in Georgia and 7.6% (6/76) in Florida are infected with *Spirometra* sp., as determined by double centrifugal fecal flotation. *Spirometra* sp. 3 plerocercoids (spargana) have been reported in a western rat snake *(Pantherophis obsoletus*) in Louisiana, an eastern racer (*Coluber constrictor*) and a western ribbon snake (*Thamnophis proximus*) in Mississippi, and three meerkats (*Suricata suricatta*) in a zoo in South Carolina, all serving as intermediate or paratenic hosts (Kuchta et al., 2021; McHale et al., 2020; Verocai et al., 2023; Waeschenbach et al., 2017). More notably, fish were not thought to be suitable hosts for *Spirometra* spp; however, 58% of killifish (*Austrolebias charrua*) in Uruguay were infected with plerocercoids belonging to *S. decipiens* complex 1 (Vettorazzi et al., 2023). While the role that fish play in the life cycle seems to be limited, this does create a shift in the historical thoughts that fish were not involved in the life cycle of *Spirometra* spp. (Kuchta et al., 2015, 2024).

Clinical Disease

Spirometriosis, or intestinal infections with adult *Spirometra* spp. tapeworms, are typically subclinical in domestic dogs and cats. However, some clinical signs such as vomiting/diarrhea and weight loss have been reported (Lillis & Burrows, 1964; Mueller, 1974).

Sparganosis, caused by infections with *Spirometra* sp. plerocercoids (spargana), is a condition where domestic cats and dogs serve as paratenic or second intermediate hosts (Buergelt et al., 1984; Drake et al., 2008; McHale et al., 2020; Woldemeskel, 2014). Infections occur after ingestion of i) procercoid infected copepods in freshwater or ii) plerocercoids through predation or scavenging of infected intermediate hosts (Bowman, 2021). The plerocercoids (spargana) can either be proliferative (asexual replication of the plerocercoids with continuous branching and budding) or non-proliferative (presence of a single larva) after ingestion (Woldemeskel, 2014). The clinical disease and prognosis can vary for both forms, but fatal outcomes are most associated with the proliferative form (Buergelt et al., 1984; Drake et al., 2008; McHale et al., 2020; Woldemeskel, 2014).

Clinical signs of sparganosis reported in domestic dogs range depending on anatomic location, from a palpable abdominal mass and distention, pneumothorax cause by nodules on the pleura and pulmonary parenchyma, and forelimb lameness (Beveridge et al., 1998; Drake et al., 2008; Simpson et al., 2012). Clinical signs reported in domestic cats include subcutaneous cyst-like masses (Woldemeskel, 2014). In general, clinical signs are associated with the area where the spargana are found. Treatment has a guarded prognosis, often requiring a combination of drug therapy to treat and surgical lavage to remove plerocercoids that are often unsuccessful (Drake et al., 2008).

Zoonosis

Sparganosis, caused by migrating *Spirometra* spp. plerocercoids (spargana), is a condition where humans become aberrant paratenic hosts. Sparganosis has been reported more than 2000 times in humans with the majority of those cases reported in China and Korea (Kuchta et al., 2021). In North America, approximately 70 human cases have been reported since sparganosis the first two cases were reported in Florida in 1908 and Texas in 1914 (McHale et al., 2020; Mueller et al., 1963). Humans are infected after i) ingestion of procercoid infected copepods from drinking contaminated freshwater, ii) ingestion of plerocercoids from infected intermediate hosts, mainly snakes and frogs, and iii) contact with infected poultices to open wounds (Kuchta et al., 2021; Waeschenbach et al., 2017). Human sparganosis is clinically seen as migrating plerocercoids (spargana) with symptoms depending on the infection location (Kuchta et al., 2021). The most common form of sparganosis reported in humans is cutaneous, but other locations such as cerebral, visceral, and ocular have also been documented (Kuchta et al., 2021; Y. Zhu et al., 2019). Cutaneous sparganosis is characterized by local erythema or nodules slowly growing under the skin mainly on the limbs, trunk, or scrotum (Kuchta et al., 2015, 2021; Q. Liu et al., 2015). Diagnosis can be achieved using a combination of neuroimaging, serological tests and accurate species identification targeting the COI gene (Kuchta et al., 2021). Treatment varies based on the form of sparganosis but can be broadly achieved through medical or surgical intervention in localized infections and high doses of praziquantel in proliferative sparganosis (Kikuchi & Maruyama, 2020; Kuchta et al., 2021).

1.2.2 Order Cyclophyllidea

Cyclophyllidean tapeworms, often referred to as true tapeworms, are a diverse group with 18 families that infects a variety of hosts including domestic dogs and cats (Families Dipylidiidae, Mesocestoididae, Taeniidae), horses, cattle, sheep and goats (Family Anplocephalidae), and birds, rodents and humans (Families Davaineidae and Hymenolepididae) (Špakulová et al., 2011). Unlike Diphyllobothriidean tapeworms that use bothria as a holdfast organ, Cyclophyllidean tapeworms have a highly specialized holdfast organ called a scolex. The scolex has four muscular suckers and a rostellum that can be retractable or non-retractable, and can also be armed (has hooks), or unarmed (no hooks). The indirect life cycles mostly involve terrestrial food chains including small vertebrates and/or arthropods.

1.2.2.1 Family Mesocestoididae

1.2.1.1.1 *Mesocestoides* spp.

Taxonomy

There are at least 27 recognized species of *Mesocestoides* (Schmidt, 1986). However, the presence of overlapping morphologic features and measurements, and the absence of molecular verification has made it difficult to definitively identify individual *Mesocestoides* spp., and therefore, most case reports do not report *Mesocestoides* to species (H. A. James, 1968; J. R. J. Jesudoss Chelladurai & Brewer, 2021; Loos-Frank, 1990). Despite these challenges, morphological descriptions and the COI gene were used to provide high statistical support for the following *Mesocestoides* spp: *M. literatus*, *M. melesi*, *M. corti/M. vogae, M. canislagopodis, M. lineatus*, and the two unnamed species groups *Mesocestoides* spp. M1 *and* Mesocestoides spp. (Mongolia) (J. R. J. Jesudoss Chelladurai & Brewer, 2021).

Morphology

<u>Egg</u>

The egg is clear, oval, thin-walled, measures about 40 -60 µm by 35 – 43 µm, and contains an oncosphere embryo with three pairs of visible hooks (Bowman, 2021).

<u>Metacestode</u>

There are two intermediate stages, the first is hypothesized to be a cysticercoid, a single non-invaginated scolex withdrawn into a small vesicle with practically no cavity. The second is a tetrathyridia, which have been described as normal or aberrant, cephalic or acephalic (Conn et al., 2011). Normal tetrathyridia have a well-developed tetra-acetabulate scolex, a solid hind body with well-organized musculature, normal excretory ducts, and normal tegument (Conn et al., 2011). Normal tetrathyridia are either free in the body cavity or individually enclosed in host-induced fibrotic capsules (Conn et al., 2011). Aberrant tetrathyridia also have a normal scolex, tegument, and excretory ducts, but have an unusually elongated body with deep convolutions and one or multiple aberrant tetrathyridia can be contained within a fibrous capsule (Conn et al., 2011). Cephalic tetrathyridia have a well-developed scolex and four suckers and acephalic tetrathyridia lack suckers (Padgett, 1991). Recently, a post-larval, pre-tetrathyridia stage was found in a skink in Oklahoma (McAllister et al., 2018). There were four different stages found, all of which have lost the hooks of the hexacanth embryo but have not formed the cercomer typically found in developing metacestode stages (McAllister et al., 2018). The four morphologies are described as: i) a slightly elongated axis with no specific features; ii) slightly larger formed showing early develops of a scolex, but absent an apical sucker; iii) larger forms with a more developed and organized scolex, a muscular apical sucker, and a single excretory bladder with a posterior excretory pore; and iv) a fully formed tetra-acetabulate scolex with well-differentiated muscular suckers, well-formed muscular apical suckers and a well-developed excretory bladder and pore posteriorly (McAllister et al., 2018).

Adult

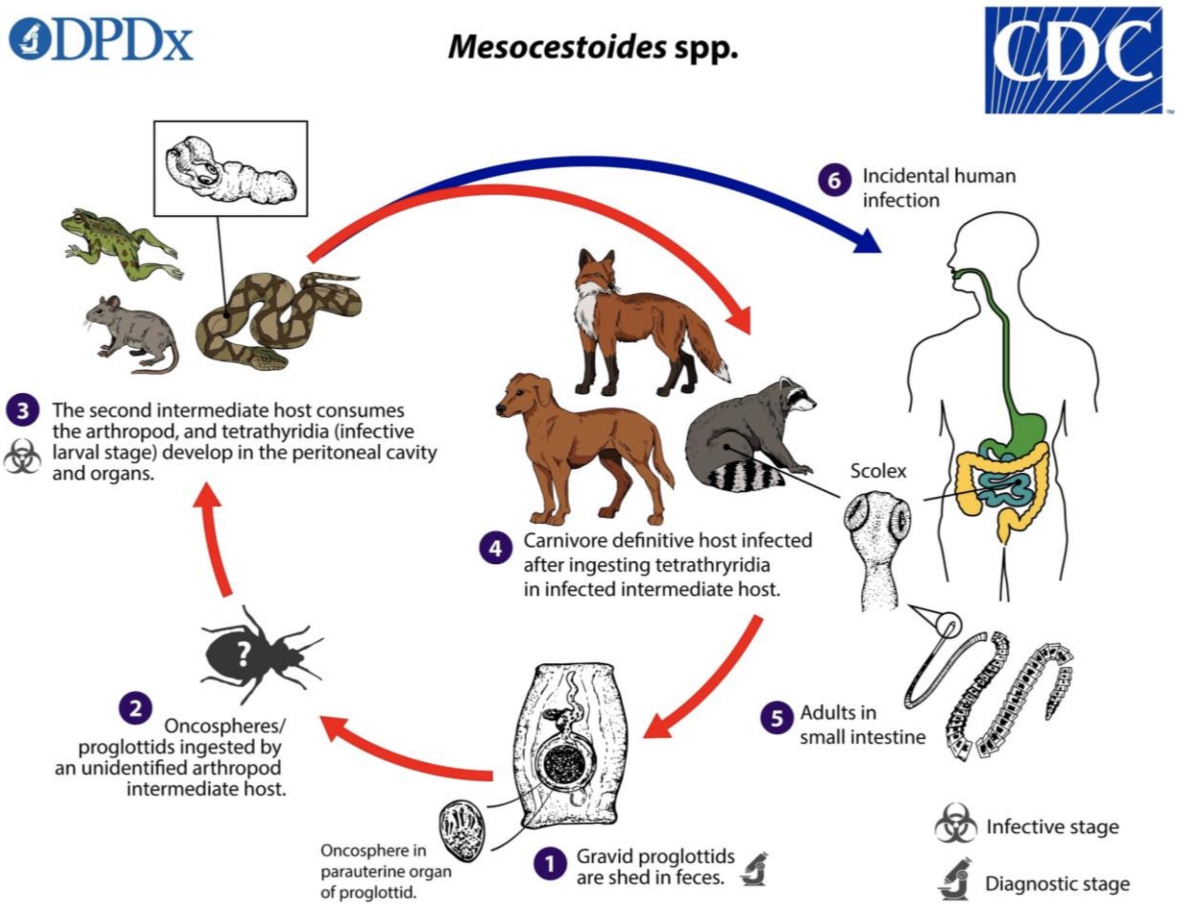
Adults of *Mesocestoides* spp. have a scolex that is slightly angular and oval shaped. Adults have four suckers but are lacking a rostellum. Immature proglottids are wider anteriorly than posteriorly with a medioventral genital pore. Gravid proglottids are longer than they are wide. The mature proglottids have one set of reproductive organs with many testes located laterally along the total length of the proglottid. The ovary is bilobed and the eggs are stored in thick-walled, slightly oval, paruterine organ measuring 530-600 µm x 440-550 µm (Cho et al., 2013).

Life Cycle

Mesocestoides spp. have a complex, and still, poorly understood life cycle (J. R. J. Jesudoss Chelladurai & Brewer, 2021; McAllister et al., 2018). There is debate over whether the life cycle requires the first intermediate host to be an arthropod or not (H. A. James, 1968; J. R. J. Jesudoss Chelladurai & Brewer, 2021; Loos-Frank, 1990). Currently, there is evidence supporting the three-host lifecycle with *Mesocestoides* DNA found in naturally infected ants and acephalic larvae found in the celomic cavity of the dung beetle *Onthophagus hecate* (H. A. James, 1968; Padgett & Boyce, 2005). However, in both studies infections could not be established in mice (H. A. James, 1968; Padgett & Boyce, 2005).

The life cycle, illustrated in Figure 1.3, begins with adults in the small intestine of definitive hosts shed eggs in the feces. Eggs are hypothesized to be ingested by a coprophagic arthropod, the first intermediate host, most likely forms a cysticercoid as the first metacestode stage (Bowman, 2021). The coprophagic arthropod is then ingested by an amphibian, rodent, reptile, or bird, as the second intermediate host, and tetrathyridia develop in the body cavity (J. R. J. Jesudoss Chelladurai & Brewer, 2021). The infected intermediate host is then ingested by the definitive host where the tetrathyridia develop into adults in the small intestine.

Figure 1.3 Life cycle of *Mesocestoides* spp. Definitive hosts include domestic dogs, red foxes (*Vulpes vulpes*), and raccoons, while intermediate hosts include an unidentified arthropod first and a reptile, rodent or amphibian second. **(CDC, 2019d)**



Prevalence

Mesocestoides spp. has been documented in a wide range of definitive hosts in North America including, domestic dogs and cats, foxes, wild canids and felids, raccoons, and opossums. *Mesocestoides* spp. has also been reported in various intermediate hosts as well including rodents, lizards, snakes, and birds. A recent study combined prevalence data reported from definitive and intermediate hosts to determine global prevalence of *Mesocestoides* spp. in North America (J. R. J. Jesudoss Chelladurai & Brewer, 2021). The overall pooled prevalence of *Mesocestoides* spp. in definitive hosts in the United States is 18% (J. R. J. Jesudoss Chelladurai

& Brewer, 2021). In North America, there was a higher pooled prevalence in wild canids and felids, 17.95% and 14.4%, respectively, than domestic dogs and cats, 0.82%, and 0.75%, respectively (J. R. J. Jesudoss Chelladurai & Brewer, 2021). In intermediate hosts, the overall pooled prevalence in intermediate hosts in North America was 7.04% (J. R. J. Jesudoss Chelladurai & Brewer, 2021). The pooled prevalence in North America is highest in amphibians (11.26%), followed by snakes (10.88%), rodents (3.92%), and lizards (3.24%) (J. R. J. Jesudoss Chelladurai & Brewer, 2021).

Clinical Disease

Intestinal infections of adult *Mesocestoides* spp. are acquired after ingestion of tetrathyridia in infected second intermediate hosts. Intestinal infections of adult *Mesocestoides* spp. area typically subclinical (McGarry et al., 2020). Owners often report visualizing motile proglottids in the feces of infected animals. Other clinical signs reported are diarrhea and ill thrift.

Peritoneal larval cestodiasis, caused by *Mesocestoides* spp., is a clinical disease that occurs when domestic dogs or cats become accidental intermediate hosts after ingestion of an infected first stage intermediate host with a first-stage larva or a second intermediate host with a tetrathyridium (Boyce et al., 2011; Dahlem et al., 2015). The disease is characterized by tetrathyridia asexually multiplying, penetrating the intestinal wall, invading the peritoneal cavity, and causing potentially life-threatening peritonitis (Conn, 1990; Montalbano Di Filippo et al., 2018; Siles-Lucas & Hemphill, 2002). Clinical signs are secondary to peritonitis and include abdominal distension, lethargy, anorexia, vomiting, urinary incontinence, and excessive urination and drinking (Boyce et al., 2011; Crosbie et al., 1998; Papini et al., 2010; Wirtherle et al., 2007; Yasur-Landau et al., 2019). The tetrathyridia have been documented in various forms within the abdomen including cephalic, larvae with an inverted scolex and four-well developed suckers, and acephalic, larvae without a scolex or suckers (Wirtherle et al., 2007). The tetrathyridia have also been found floating in abdominal fluid and/or enclosed in small cysts (Wirtherle et al., 2007). Prognosis and survival of the infected animal is significantly influenced by the severity of clinical signs (Boyce et al., 2011). Diagnosis involves a combination of abdominal ultrasonography, abdominocentesis, and a laparotomy followed by morphological and molecular identification of parasite larva found (Carta et al., 2021). These rare cases of canine and feline peritoneal larval cestodiasis can be difficult to treat and there is no treatment available that can completely eradicate or prevent reinfection of the disease (Carta et al., 2021). Tetrathyridia can be removed from the abdomen by closed peritoneal lavage using a large bore needle or catheter when free-floating in the abdomen or when found in cysts (Boyce et al., 2011). Lavage can be followed by fenbendazole or praziquantel, but ultimately prolonged treatment of fenbendazole at high doses is the most effective method (Carta et al., 2021).

Zoonosis

Human infections with adult *Mesocestoides* spp. have been reported worldwide (Fuentes et al., 2003). *Mesocestoides variabilis* adults have been reported, in low numbers, from the United States. Infections are clinically characterized by non-specific and recurrent gastrointestinal signs. There have been no reports of peritoneal larval cestodiasis in humans (CDC, 2019e).

1.2.2.2 Family Dipyliididae

1.2.2.2.1 *Dipylidium caninum*

Taxonomy

Dipylidium caninum, also known as the flea tapeworm, double-pored tapeworm, or cucumber seed tapeworm, is ubiquitous and the most common tapeworm of domestic dogs and cats in North America. The genus *Dipylidium* was thought to be monotypic, with only one recognized species. However, recent studies have suggested that may not be true. Phylogenetic analysis of the partial mitochondrial 12S gene and the 28S genes showed genetic differences between *D. caninum* infections of domestic dogs and cats (Beugnet et al., 2014; Low et al., 2017). Furthermore, Jesudoss Chelladurai et al. provided the entire genome sequence of the feline strain and used benchmarking universal single-copy orthologs (BUSCO) to delineate that the canine and feline strains are two distinct species of *D. caninum* (J. R. J. Jesudoss Chelladurai et al., 2023). The genotype differences were solidified when an in vivo experimental study showed biological adaptation with shorter prepatent periods and longer life spans when the canine or feline genotype infected the corresponding host compared to cross-infections (Beugnet et al., 2018).

Morphology

Egg

Eggs are clustered together in packets of no more than 29 eggs (Bowman, 2021). Egg packets are formed from outer pockets of the uterus and measure 120 – 200 µm with individual eggs measuring approximately 30 µm. Each individual egg contains a hexacanth embryo with 3 pairs of hooks (Bowman, 2021; Oehm et al., 2024).

Metacestode

There is only one metacestode stage for D. caninum, a cysticercoid. It is a single, non-invaginated scolex withdrawn into a small vesicle with no cavity (Soulsby, 1968).

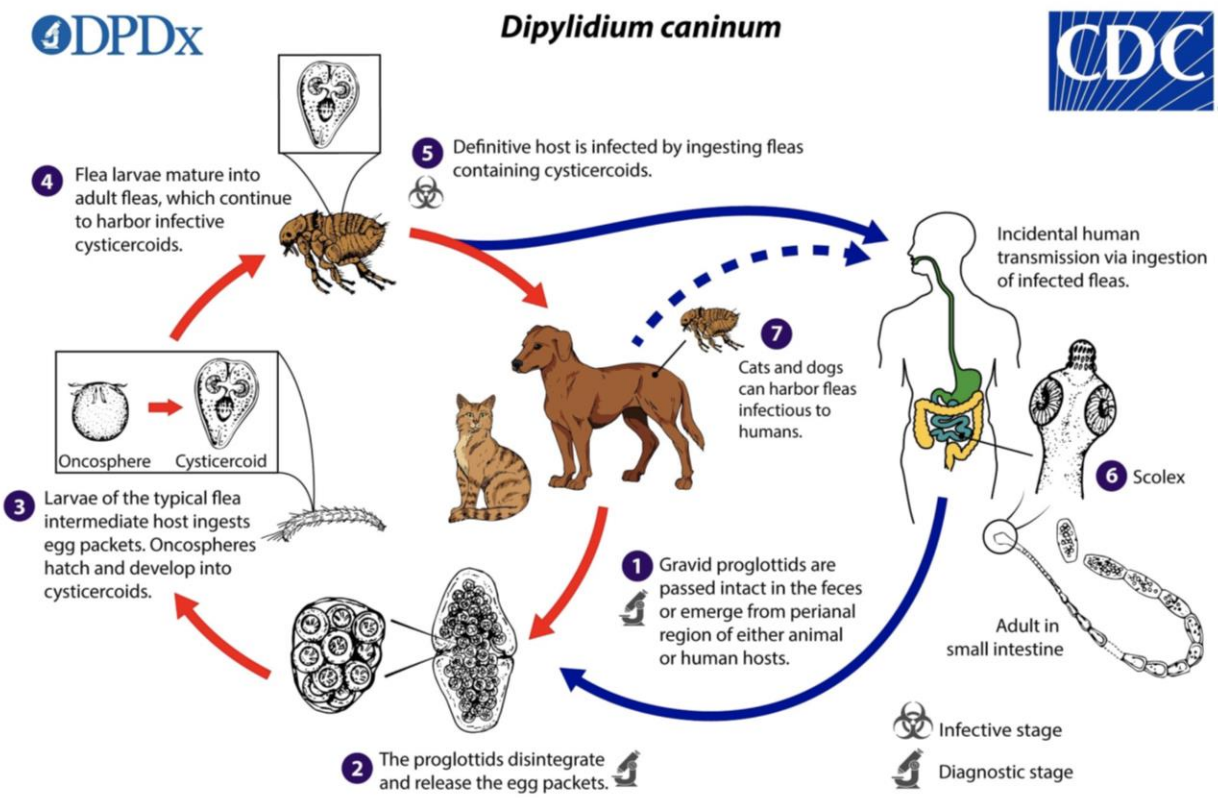
Adult

Adults of *D. caninum* have a scolex with four suckers and a retractable, armed rostellum with 3-4 rows of thorn-shaped hooks (Bowman, 2021; Oehm et al., 2024). Mature proglottids have two sets of reproductive organs each set located laterally in the proglottid near the bilateral genital pores. The ovaries are paired and there are many testes. Mature, gravid proglottids found at the posterior end of the tapeworm are white and tapered on both ends resembling cucumber seeds (Oehm et al., 2024).

Life Cycle

The indirect life cycle, as illustrated in Figure 1.4, begins when gravid proglottids are shed in the feces. When fresh proglottids are shed in the feces, they move by contracting and relaxing to empty egg packets into the environment. The egg packets are ingested by *Ctenocephalides felis* (the cat flea), *Trichodectes canis* (the canine biting louse), or rarely *Ctenocephalides canis* (the dog flea) in the environment, and a cysticercoid develops. Of the three, *C. felis* is considered to be the most common intermediate host in the US. The infected arthropod is then ingested, and the cysticercoid evaginates, attaches to the intestinal wall, and develops into an adult in the small intestine of domestic dogs and cats. The infection becomes patent and proglottids are shed in the feces 14 – 21 days post-infection (Soulsby, 1982).

Figure 1.4 Life cycle of *Dipylidium caninum*. Definitive hosts include domestic dogs and cats, while the intermediate host is *Ctenocephalides felis*, the cat flea. (CDC, 2019b)



Prevalence

Dipylidium caninum infection is likely found wherever the primary intermediate host, *Ctenocephalides felis*, is found and prevalence varies depending on diagnostic test used (C. B. Adolph & Peregrine, 2021; Rousseau et al., 2022). Prevalence of *D. caninum* infection in dogs varies with reports being as low as 4.0% or as high as 60% in domestic dogs and 1.8% to 52.7% in domestic cats (CAPC, 2025). A study evaluating the prevalence of gastrointestinal parasites through necropsy in shelter cats in Oklahoma, USA revealed that 40/116 (34.5%) of shelter cats were positive for *D. caninum* (Little et al., 2015). In Georgia, a study analyzing the feces of shelter cats using a centrifugal fecal flotation with Sheather's sugar only found 1/103 (1.0%) of shelter cats positive for *D. caninum* infection (Hoggard et al., 2019). In Florida, a study also analyzing the feces of shelter cats using a centrifugal fecal flotation with Sheather's sugar found

0/76 of the shelter cats infected with *D. caninum* (Wyrosdick et al., 2017). A study in shelter dogs in Oklahoma also revealed that 48/97 (49.5%) of shelter dogs were infected with *D. caninum* adults at necropsy, when only 3/48 (6.3%) of those positive dogs at necropsy also positive for egg packets on fecal flotation (C. Adolph et al., 2017).

A study analyzing parasite prevalence in client owned-cats in Oklahoma over a 12 year period revealed that *D. caninum* proglottids and eggs were found in 29/2586 (1.1%) of fecal samples (Nagamori et al., 2020b). Similarly, the same study was performed in client-owned dogs and *D. caninum* proglottids and eggs were found in 62/7,409 (0.84%) of fecal samples (Nagamori et al., 2020a).

Clinical Disease

The most common clinical sign reported in infected domestic animals is scooting due to perianal irritation from proglottid movement (Saini et al., 2016). It is also not uncommon to also see proglottids in the perianal region, stuck on fur on the hind legs, or moving on fresh feces of infected animals.

Zoonosis

Humans dipylidiasis is rare in the United States with less than 100 case reports since it was first reported in 1903 (Molina et al., 2003; Stiles, 1903). Human dipylidiasis is a clinical disease that occurs when humans, especially children, accidentally ingest the infected arthropod intermediate host and the adult tapeworm develops in the intestines. Similar to domestic dogs and cats, proglottids can be found in the feces of infected humans. Infections are self-limiting unless there is repeated environmental exposure. Praziquantel and niclosamide have also been effective in treating infections in humans (Molina et al., 2003). In human dipylidiasis, house pets serve as the main source of infection for both *D. caninum* and the arthropod intermediate host. To prevent recurrent human infections, house pets should be treated for both *D. caninum,* eliminating the production of gravid proglottids, and fleas, interrupting the tapeworm life cycle (Molina et al., 2003).

1.2.2.3 Family Taeniidae

Tapeworms belonging to the Taeniidae family are unique in that they all produce a morphologically similar eggs commonly referred to as "taeniid eggs" or "taenia-type egg". Therefore, when taenia-type eggs are found in the feces of infected domestic dogs and cats, in the absence of a proglottid, morphologic identification can only be to the family level. In order to confirm identity past the family level, molecular confirmation is needed. This is important since some taeniid tapeworms are zoonotic, i.e. *Taenia multiceps* and *Echinococcus* spp..

1.2.2.3.1 *Taenia spp.*

Taxonomy

Taenia spp. tapeworms have been found worldwide and the metacestode stages can cause significant economic loss in the intermediate hosts in endemic areas (Varcasia et al., 2022; Wang et al., 2021). There are multiple *Taenia* spp. that can infect both domestic cats and dogs as definitive hosts (see Table 1.1). The main species that infects dogs is *Taenia pisiformis*, but *T. hydatigena*, *T. ovis*, *T. serialis*, and *T. multiceps* have also been reported. The main species that infects domestic cats is *T. taeniaeformis*.

Table 1.1. Species of *Taenia* that infect Domestic Dogs and Cats in North America

Taenia spp.	Distribution	Definitive Host	Intermediate Host	Metacestode Stage	Zoonosis
T. taeniaeformis	Worldwide	Domestic cats	Mice and rats	Strobilocercus	None
T. pisiformis	Worldwide	Domestic dogs	Cottontail rabbits	Cysticercus	None
T. crassiceps	Worldwide	Dog, wild canids	Rodents	Cysticercus	Yes
T. hydatigena	Worldwide	Domestic dogs	Mainly sheep	Cysticercus	None

T. ovis	Worldwide	Domestic dogs	Mainly sheep	Cysticercus	None
T. serialis	Worldwide	Domestic dogs	Cottontail rabbits	Coenurus	None
T. multiceps	Absent in US and New Zealand	Domestic dogs	Mainly sheep	Coenurus	Yes

Morphology

<u>Egg</u>

Eggs produced by *Taenia* spp. are morphologically similar to other taeniid tapeworms including *Echinococcus* spp. Taenia-type eggs are spherical to ellipsoidal and range from 30 – 50 µm in size. The outer shell is passively removed before the egg is freed into the environment. The taenia-type eggs have an oncosphere larva, that is immediately infectious, with three pairs of hooks contained in a thick, impermeable embryopore made up of a keratin-like protein. (R. C. A. Thompson, 2017).

<u>Metacestode</u>

The metacestode stages of *Taenia* spp. can be found in one of three distinct stages a cysticercus, strobilocercus, or coenurus. A cysticercus has a single bladder with an evaginated protoscolex, which is a juvenile scolex formed from the germinal layer of a metacestode (C. B. Adolph & Peregrine, 2021). A strobilocercus has an elongated body with an evaginated protoscolex. A coenurus has a single bladder with many protoscolices; each capable of maturing into a tapeworm (Soulsby, 1982).

<u>Adult</u>

Taenia spp. adult tapeworms are morphologically similar between species. Strobila are long, ranging from a few centimeters to a few meters in length, and have numerous proglottids that vary in size between species (Bowman, 2021; Jones & Pybus, 2000). On the anterior end, there is a scolex with four acetabulate suckers and an armed rostellum (Jones & Pybus, 2000).

The rostellum has two circular rows of hooks, one circle has large hooks and the other circle has small hooks and the number and length of the hooks can be used to identify *Taenia* spp. (Table 1.2) (Bowman, 2021; Loos-Frank, 2000). Mature proglottids have one set of reproductive organs and with irregularly alternating unilateral genital pores. Mature proglottids have numerous testes situated in one or more horizontal layers. The ovary is bilobed and located posterior to the testes. The compact vitelline gland is located posterior to the ovary. The uterus has a longitudinal median stem with multiple bilateral branches (Jones & Pybus, 2000).

Table 1.2 Number and Size of rostellar hooks found in *Taenia* spp. that infect dogs and cats in North America **(Bowman, 2021; Loos-Frank, 1990)**.

***Taenia* spp.**	**Number of hooks**	**Large hooks (μm)**	**Small hooks (μm)**
T. taeniaeformis	26-52	300-450	187-293
T. pisiformis	32-48	220-294	114-117
T. crassiceps	28-34	172-200	121-155
T. hydatigena	26-44	170-226	110-160
T. ovis	22-36	137-195	84-141
T. serialis	22-34	110-177	85-160
T. multiceps	22-34	120-180	73-160

Life Cycle

The typical *Taenia* spp. life cycle begins when taenia-type eggs (thick, striated, brown shelled eggs) are shed in gravid proglottids in the feces of the infected animal. *Taenia* spp. has an indirect lifecycle requiring intermediate hosts that differ depending on species (Table 1.1). A suitable intermediate host ingests taenia-type eggs where one of the three distinct intermediate metacestode stages develops. The infected intermediate host is predated upon by the definitive host and the metacestode develops into an adult in the small intestine.

Prevalence

Taenia spp. are a group of tapeworms with worldwide distribution with infections in domestic dogs and cats most likely being underestimated based off necropsy studies (C. B. Adolph & Peregrine, 2021). Infections are higher in shelter dogs than client-owned dogs seemingly due to the administration of broad-spectrum monthly preventatives in client-owned dogs (C. Adolph et al., 2017). A necropsy study evaluating the prevalence of gastrointestinal parasites in shelter cats at an animal shelter in Oklahoma, USA revealed that 30/116 (25.9%) of shelter cats were positive for *Taenia taeniaeformis* (Little et al., 2015). A study in shelter dogs in Oklahoma also showed that 7/97 (7.2%) of shelter dogs were infected with *Taenia* spp. adults at necropsy (C. Adolph et al., 2017).

A study analyzing parasite prevalence in client owned-cats in Oklahoma over a 12 year period revealed that taeniid proglottids and eggs were found in 30/2586 (1.2%) of fecal samples (Nagamori et al., 2020b). Similarly, the same study was performed in client-owned dogs and taeniid proglottids and eggs were found in 35/7,409 (0.47%) of fecal samples (Nagamori et al., 2020a)

Clinical Disease

Intestinal infections of domestic dogs and cats typically do not cause clinical disease; however, vomiting/diarrhea, lethargy, weight loss, and intestinal obstruction have been seen (Bowman, 2021; Wilcox et al., 2009).

Larval infections with *Taenia pisiformis* have been reported in rabbits and hares after the ingestion of eggs from the environment. The metacestode stage, cysticercus, develops and infects the liver capsule, mesentery, and greater omentum (Stancampiano et al., 2019; Wang et al., 2021). This condition greatly affects breeding rabbits, especially in China (Wang et al., 2021). Clinical signs are usually asymptomatic, but heavy infections can lead to intestinal obstruction, abdominal discomfort, severe hepatitis, and emaciation leading to economic losses in slaughterhouses (Wang et al., 2021).

Cerebral coenurosis, caused by *Taenia serialis*, occurs after accidental ingestion of eggs from contaminated feces (Jull et al., 2012). It is proposed that the oncosphere larva goes through aberrant larval migration to the brain to cause cerebral coenurosis (Huss et al., 1994; Jull et al., 2012). This condition has sporadically been reported in domestic cats in Australia, North America, and the United Kingdom (Jull et al., 2012; Slocombe et al., 1989). Clinical signs are typically not seen in small cysts that are alive because the cysts evoke a small or minimal inflammatory response (Jull et al., 2012). An inflammatory response is normally evoked when cysts start to degenerate and neurological signs develop and deterioration occurs over a course of one to two weeks (Jull et al., 2012; Mahanty & Garcia, 2010). Diagnostic imaging such as magnetic resonance imaging (MRI) and computed tomography (CT) can be used to identify cysts caused by cerebral coenurosis (Jull et al., 2012). While CT is better suited for visualizing calcified lesions, MRI is a more sensitive and specific diagnostic tool when diagnosing cerebral coenurosis in domestic cats (Jull et al., 2012; Nash et al., 2006). Treatment studies in domestic cats are not available. However, treatment can be adapted from human coenurus cases where medical management can be effective in early small lesions and surgical intervention on accessible cysts (Jull et al., 2012). Despite this suggestion all cerebral coenurosis cases in domestic cats to date have been fatal (Jull et al., 2012).

Zoonosis

Human coenurus, caused by *Taenia serialis* in North America, occurs in humans after accidental ingestion of eggs after contact with infected feces or contaminated and unwashed fruits and vegetables (Varcasia et al., 2022). Human coenurus was first reported in Africa in 1913, and since then approximately 100 cases have been reported worldwide, with six of those cases coming from North America (Ing et al., 1998; Varcasia et al., 2022). Commonly referred to as sturdy or gid, after ingestion of eggs, oncospheres hatch, penetrate intestines, and travel to the brain, spinal cord or eyes (Varcasia et al., 2022). Clinical signs include seizures, headaches, vomiting and papilledema (swelling of the optic disc) (Varcasia et al., 2022). This condition can present as giant cysts mimicking other cysticerci cysts or hydatid cysts and go undiagnosed (Varcasia et al., 2022).

Human cysticercosis, caused by *Taenia crassiceps*, is acquired after ingestion of infectious taenia-type eggs from contaminated food or water (Deplazes et al., 2019). Mainly a tapeworm found in red foxes, there are only 12 documented cases of *T. cassiceps* infection in humans worldwide with most of them occurring in immunocompromised individuals. Additionally, four of those individuals were from North America with 1/4 being from Canada. Most infections involve the subcutaneous or muscle tissue and cysticerci have been reportedly found in a hematoma in the right temple, in the shoulder, intercerebellar, and intraocular infections . Treatment normally involves a combination of praziquantel and albendazole (Deplazes et al., 2019; Ntoukas et al., 2013).

It is important to note, humans are definitive hosts for two *Taenia* spp. tapeworms, *Taenia solium*, the pork tapeworm, and *Taenia saginata*, the beef tapeworm. However, theses tapeworms are no associated with infections in domestic dogs and cats. Human taeniasis, caused by *T. solium*, occurs in the United States when undercook pork containing the cysticerci is consumed and adults develop in the small intestine of the infected human host (Sorvillo et al., 2007). Neurocysticercosis develops when eggs are ingested from contaminated food or water and the larva invade the tissue of the central nervous system (Sorvillo et al., 2007). Human taeniasis, caused by *T. saginata*, occurs after the consumption of raw or undercooked bovine meat or offal containing the cysticerci and the adults develop in the intestines of the infected human host (Braae et al., 2018).

***Echinococcus* spp.**

Echinococcus spp. are zoonotic tapeworms that exist in a predator-prey lifecycle. Like *Taenia* spp., *Echinococcus* spp. produce the same taenia-type eggs that are indistinguishable beyond the family level. Unlike *Taenia* spp., *Echinococcus* spp. adults are microscopic and their metacestode stages can infect domestic dogs, cats, and humans with potentially fatal outcomes if untreated. There are currently nine recognized *Echinococcus* spp. that occur throughout the world (Romig et al., 2017), but *Echinococcus granulosus* sensu lato and *Echinococcus multilocularis* are the two species that are found globally and are a major public health threat.

1.2.2.3.2 *Echinococcus granulosus* sensu lato

Taxonomy

Echinococcus granulosus sensu lato (s.l.), “dangerous dog tapeworm” or “dwarf dog tapeworm”, is a genotypic cluster of zoonotic tapeworms that infects domestic dogs, wild canids and lions as definitive hosts (see Table 1.3). The predominate genotypes found in North America are *Echinococcus granulosus* sensu stricto (s.s.) sheep strains (G1-G3) found mainly in domestic life cycles involving domestic dog definitive hosts and sheep intermediate hosts (R. C. A. Thompson, 2017). *Echinococcus granulosus canadensis* (cervid strains G8 and G10) are found mainly in sylvatic life cycles where wild canids serve as definitive hosts and large ungulates (i.e. elk and moose) serve as intermediate hosts (R. C. A. Thompson, 2017).

Table 1.3. Species, Strains, and Genotypes of *Echinococcus granulosus* sensu lato

Species/Strain/Genotype	Distribution	Definitive Host	Intermediate Host	Zoonosis
Echinococcus granulosus s.s./sheep (and buffalo) strains/G1-G3	Worldwide	Domestic dog, wild canids	Sheep, cattle, buffalo, goat, camel, yak	CE
Echinococcus equinus/horse strain/G4	Africa, Europe, Middle East, USA	Domestic dog	Horse, donkey, zebra	Not reported
Echinococcus ortleppi/cattle/G5	Africa, Asia, Central & South America, Europe	Domestic dog	Cattle, buffalo, goat, sheep	CE
Echinococcus canadensis cluster Camel strain/G6	Africa, Asia, South America	Domestic dog	Camel, cattle, goat, sheep	CE
Pig strain/G7	Worldwide focally	Domestic dog	Pig, wild boar, (cattle)	CE
Cervid strains/G8, G10	Eurasia, North America	Domestic dog, wild canids	Moose, reindeer, wapiti	CE
Echinococcus felidis/lion strain	Africa	Lion	Warthog	Not reported

CE = Cystic Echinococcosis

Morphology

Egg

Eggs produced by *Echinococcus granulosus* are morphologically similar to other taeniid tapeworms including *Taenia* spp. Taenia-type eggs are spherical to ellipsoidal and range from 30 – 50 µm in size. The outer shell is passively removed before the egg is freed into the environment. The taenia-type eggs have an oncosphere larva, that is immediately infectious, with three pairs of hooks contained in a thick, impermeable embryopore made up of a keratin-like protein (R. C. A. Thompson, 2017).

Metacestode

The metacestode stage is a unilocular, subspherical, fluid-filled mass called a unilocular hydatid cyst (R. C. A. Thompson, 2017). The cysts consist of an inner germinal layer where brood capsules form via asexual replication. The protoscolices form inside the brood capsule from the germinal layer. There is an acellular laminal layer that is external, tough and elastic. The cyst is then surrounded by a host-induced fibrous layer called the adventitial layer (R. C. A. Thompson, 2017). Unilocular hydatid cysts can be fertile containing protoscolices, brood capsules, and calcareous corpuscles, called hydatid sand. Unilocular cysts can also not be fertile and just contain cyst fluid (Thompson, 1983, Deplazes, 2016).

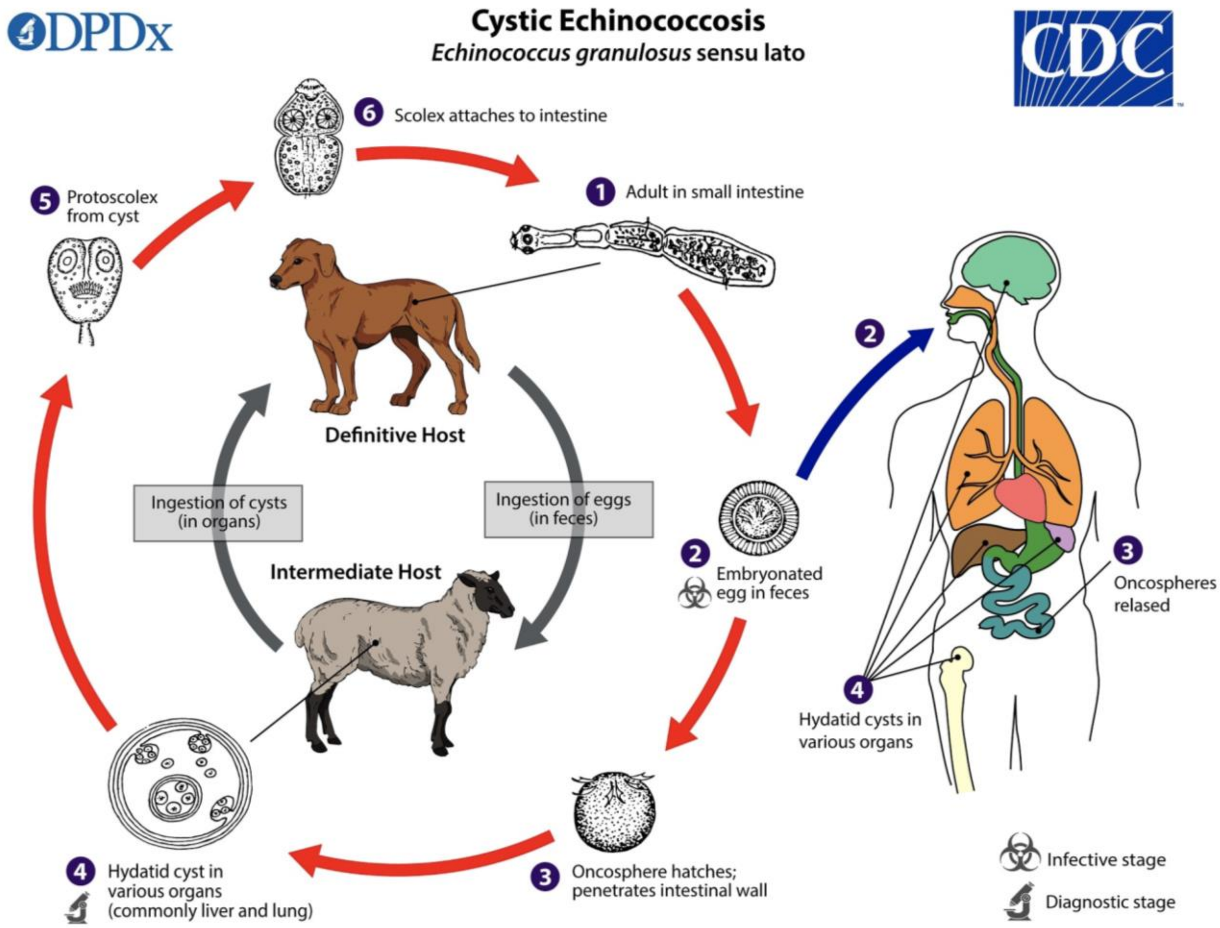
<u>Adult</u>

Adults of *E. granulosus* are 2-7 mm in length and usually made up of only 3-5 segments (R. C. A. Thompson, 2017). Adults also have a scolex, with four suckers and an armed rostellum with two rows of hooks. Mature proglottids have one set of reproductive organs including 20-50 testes located laterally to the ovary and uterus. The ovary has two branches and a medially located uterus with lateral branches. The genital pore is unilateral and located in the middle or posterior to the middle of the proglottid. Adults of *E. granulosus* s.l. can produce and detach new proglottids every 7 – 14 days (R. C. A. Thompson, 2017).

<u>Life Cycle</u>

The predator-prey lifecycle, as illustrated in Figure 1.5, begins when taenia-type eggs are shed in the feces of infected animals into the environment. Eggs ingested by sheep or large ungulate intermediate hosts, and the oncosphere larvae develop into unilocular hydatid cysts in the liver and lungs of infected animals (Romig et al., 2017). A domestic dog or wild canid definitive host ingests unilocular hydatid cysts either through predation or ingestion of offal (infected organs) (R. C. A. Thompson, 2017). Adults mature in the small intestines and can produce taenia-type eggs in the feces 34 – 58 days post infection (R. C. A. Thompson, 2017).

Figure 1.5 Life cycle of *Echinococcus granulosus* sensu lato. Canid definitive hosts include domestic dogs, coyotes (*Canis latrans*) and wolves (*Canis lupus*), while ungulate intermediate hosts include domestic sheep, moose, and elk (CDC, 2019c).



Prevalence

The global disease burden is still unknown largely due to poor disease surveillance, which hinders proper disease reporting (De La Cruz-Saldana et al., 2024). North America is not immune to this global issue, and the prevalence is not known here either.

In Canada, *E. granulosus* s.s. sheep and buffalo strains, G1 and G3, respectively, are absent from livestock species (Priest et al., 2021). *Echinococcus canadensis* cervid strains G8 and G10 however, have been found in all Canadian provinces west of the Maritimes (Nova Scotia, Prince Edward Island and New Brunswick) and south of the high arctic (J. Schurer et al., 2013). It was thought that all *Echinococcus* spp. were free from the eastern Canadian provinces due to the areas being historically free of wolves (Deplazes et al., 2017; Sweatman, 1952). However, the absence of wolves has granted the movement and expansion of coyotes into eastern Canada and the Northeastern United States from the Great Lakes region of Southern Canada and Northcentral United States (Priest et al., 2021). Adults of *E. canadensis* were found in 1/262 coyotes, and unilocular hydatid cysts were found in the lungs and liver of 4/8 moose in Nova Scotia (Priest et al., 2021). These were the first reports of any *Echinococcus* spp. in eastern Canada.

In the United States, a recent study in Wyoming found 63.6% of gray wolves, 0.55% of coyotes, and 0% of red foxes were positive for *E. granulosus* s.l. Studies from Alaska found 30% of wild canids and 34% of moose infected (Pipas et al., 2021). In California, 1.3% of the deer population in an established coyote/deer sylvatic cycle were infected (Cerda et al., 2018). Other reports of *E. granulosus* s.l. infections in the Unites States from Minnesota, Montana, Idaho, and a moose in Maine have been reported (Cerda et al., 2018). Recently, in a retrospective study analyzing banked elk tissues from re-established populations in the Great Smoky Mountains and North Cumberland Wildlife Management in Tennessee, *E. canadensis* were found in 4/253 (1.5%) of elk (*Cervus canadensis*) that were translocated from Alberta, Canada (Dell et al., 2020). A list of case reports for *E. granulosus* in wild and domestic canids and humans in the United States, can be found in Appendix A.

Clinical Disease

Echinococcosis, caused by *Echinococcus granulosus*, occurs when adults live in the small intestine of infected animals. Infection is acquired after ingestion of unilocular hydatid cysts containing protoscolices that develop to adults in the small intestine. Intestinal infection typically does not cause disease as domestic dogs are the natural definitive host.

Cystic echinococcosis (CE), caused by *Echinococcus granulosus* sheep strain (G1, G3), occurs when domestic dogs and cats serve as aberrant intermediate hosts. This condition occurs after accidental ingestion of eggs in the environment from coprophagy (Bonelli et al., 2018). After ingestion of eggs, fertile (containing protoscolices) or infertile (containing no protoscolices) unilocular hydatid cysts develop slowly, mimicking a space occupying lesion in the liver and lungs and infected animals. Due to the slow-growing nature, unilocular hydatid cysts are not discovered until years after ingestion first occurred. Surgical removal of cysts is the gold standard of treatment for CE in domestic dogs (Woolsey & Miller, 2021).

Domestic cats are not suitable definitive hosts for *E. granulosus* s.l. However, there have been reports of CE in domestic cats. Cystic Echinococcosis caused by *E. granulosus* s.s. was reported in Russia and Uruguay, and CE caused by *E. granulosus* s.l. was reported in Turkey (Armua-Fernandez et al., 2014; Burgu et al., 2004; Konyaev et al., 2012). All cases described multiple, non-painful, unilocular hydatid cysts in the abdominal cavity that were successfully treated with surgical removal unilocular hydatid cyst (Armua-Fernandez et al., 2014; Burgu et al., 2004; Konyaev et al., 2012).

Cystic echinococcosis (CE), caused by *E. granulosus* s.s. sheep strain (G1-G3), occurs when sheep intermediate hosts accidentally ingest eggs from the environment. Infected animals can exhibit clinical signs, with older animals having a higher burden of cysts than younger animals (Romig et al., 2017). The economic burden is apparent when multiple organs or whole carcasses are condemned due to unilocular hydatid cysts. *Echinococcus gransulosus* s.l. causes an estimated $3 billion dollars in food animal production losses, globally (De La Cruz-Saldana et al., 2024).

Zoonosis

Cystic echinococcosis (CE), caused by *Echinococcus granulosus* sheep strain (G1-G3), occurs in humans after accidental ingestion of infectious eggs from the environment (WHO, 2021). The disease process is similar to domestic dogs and cats diagnosed with CE (R. C. A. Thompson, 2017). The slow growing unilocular hydatid cysts can vary in size from 1 – 15 cm and be fertile (have protoscolices) or sterile (no protoscolices) (Eckert & Deplazes, 2004). Cysts can also rupture in the abdomen and release protoscolices or small cysts that can grow to larger cysts (Eckert & Deplazes, 2004).

The risk factors for human CE are i) large sheep and dog populations, ii) dogs that have access to dead livestock or offal (infected organs), iii) low availability or acceptance of cestode treatment in dogs, iv) low socioeconomic communities (Cardona & Carmena, 2013). Diagnosis is made using a combination of advanced imaging and serological tests (WHO, 2021). Treatment is difficult, expensive, and often extensive and prolonged (WHO, 2021). WHO recognizes four treatment options: i) PAIR (puncture, aspiration, injection, and re-aspiration) technique; ii) surgery; iii) drug treatment to inhibit the spread of cysts, and iv) “watch and wait”.

Human CE is considered a neglected zoonotic disease (NZD) and represents a substantial disease burden worldwide with an excess of 1 million people living with this disease at one time (WHO, 2021). Cystic echinococcosis causes an estimated 19,300 deaths worldwide and around 871,000 disability-adjusted life-years (DALYs), which are one lost year of healthy life, globally each year (WHO, 2021). Unfortunately, this disease can be very difficult to treat with 6.5% of cases relapsing after interventions and 2.2% of surgical cases ending in death (WHO, 2021).

Prevention and Control Strategies in Intermediate and Definitive Hosts

The transmission of *E. granulosus* sheep strain is dependent upon wild or domestic canids harboring adult tapeworms, domestic or wild herbivores infected with the metacestode stages, and eggs in the environment. The most effective way to reduce active transmission is to reduce the environmental contamination of eggs by removing or reducing the worm burden in domestic dogs (P. S. Craig et al., 2017). Successful and effective control programs for *E. granuslosus* should be multifaceted targeting definitive hosts with four key components: i) regular deworming of domestic dogs, ii) improved management of infected viscera (offal), iii) dog population control, and iv) health education programs (P. S. Craig et al., 2017; WHO, 2021). This program has proven to be successful on a large scale when Iceland eradicated *E. granulosus* in the 1970s (Saarma et al., 2023). On the Falkland Islands in Cyprus, a five-year program targeted educating school-aged children, housewives, and dog owners. In addition, dogs were screened and infected dogs were euthanized or given arecoline bromide. After three years the prevalence in dogs decreased from 50% to 6.8% (De La Cruz-Saldana et al., 2024). In Utah, USA a decade-long control program involved the four main components plus increased diagnostic testing options for humans and domestic dogs. These efforts decreased domestic dog infections from 28% to 1%, although, a year later, infections did rise to 10% due to owner compliance (De La Cruz-Saldana et al., 2024).

1.2.2.3.3 Echinococcus multilocularis

Taxonomy

Echinococcus multilocularis is a zoonotic tapeworm that infects domestic dogs and cats, wild canids, and humans (R. C. A. Thompson, 2017). Historically, *E. multilocularis* was divided into subspecies based on geographic location, *E. m. multilocularis* in Europe, *E. m. sibiricensis* in Alaska, and *E. m. kasakhensis* in Kazakhstan (Nakao et al., 2009a). However, genetic variation in the mitochondrial gene haplotypes has designated 18 haplotypes from 76 geographic isolates: Asian haplotypes (A1-A10), European haplotypes (E1-E5), North American haplotypes (N1 and N2), and an Inner Mongolian haplotype (O1) (Nakao et al., 2009a). A distinctive EmsB microsatellite profile, a type of genotyping that uses capillary electrophoresis to analyze complex PCR profiles, was also found in wildlife (Knapp et al., 2007). There have been additional European haplotypes of *E. multilocularis* found in the Canadian NCR including BC1 haplotype in British Columbia (K. Gesy, Hill, et al., 2013; K. M. Gesy & Jenkins, 2015; E. J. Jenkins et al., 2012; Massolo et al., 2019).

A TCS haplotype network, using the *nad2* gene in Figure 6 and the *cob* gene in Figure 7, was created to show the relationship between known haplotypes (Conlon et al., 2023; K. M. Gesy & Jenkins, 2015; Laurimäe et al., 2020; Nakao et al., 2009b). The number of distinct haplotypes recognized when comparing relationships using the *nad2* gene are 11 (Figure 6). The European, Asian and North American haplotypes are all grouped together. The Austria-E1 haplotype includes France-E2-E4, Canadian haplotypes AB1, BC1, MC3 and SK1, Poland haplotypes EmPL6 and EmPL10 along with canine samples from two Missouri case reports, plus the four representative samples from this study, C-KS-52 and 53 and C-MO-16 and 42. The Slovakia-E5 haplotype contains Poland haplotype EmPL14, and 1 isolate from Virginia and 3 isolates from New York, E316, E320 and E321. The Asian haplotypes from Kyrgyzstan is grouped with Kazakhstan-A-1-A2. The Canadian haplotypes SK5 and Sk2 are grouped with the North American N2 haplotypes from Indiana and South Dakota. The Canadian SK4 haplotype is grouped with Canadian haplotype SK3. The haplotype from Mongolia, China shows a distant relationship to the other sequences. All representative sequences with GenBank accessions can be found in Table 5.8 in Appendix C.

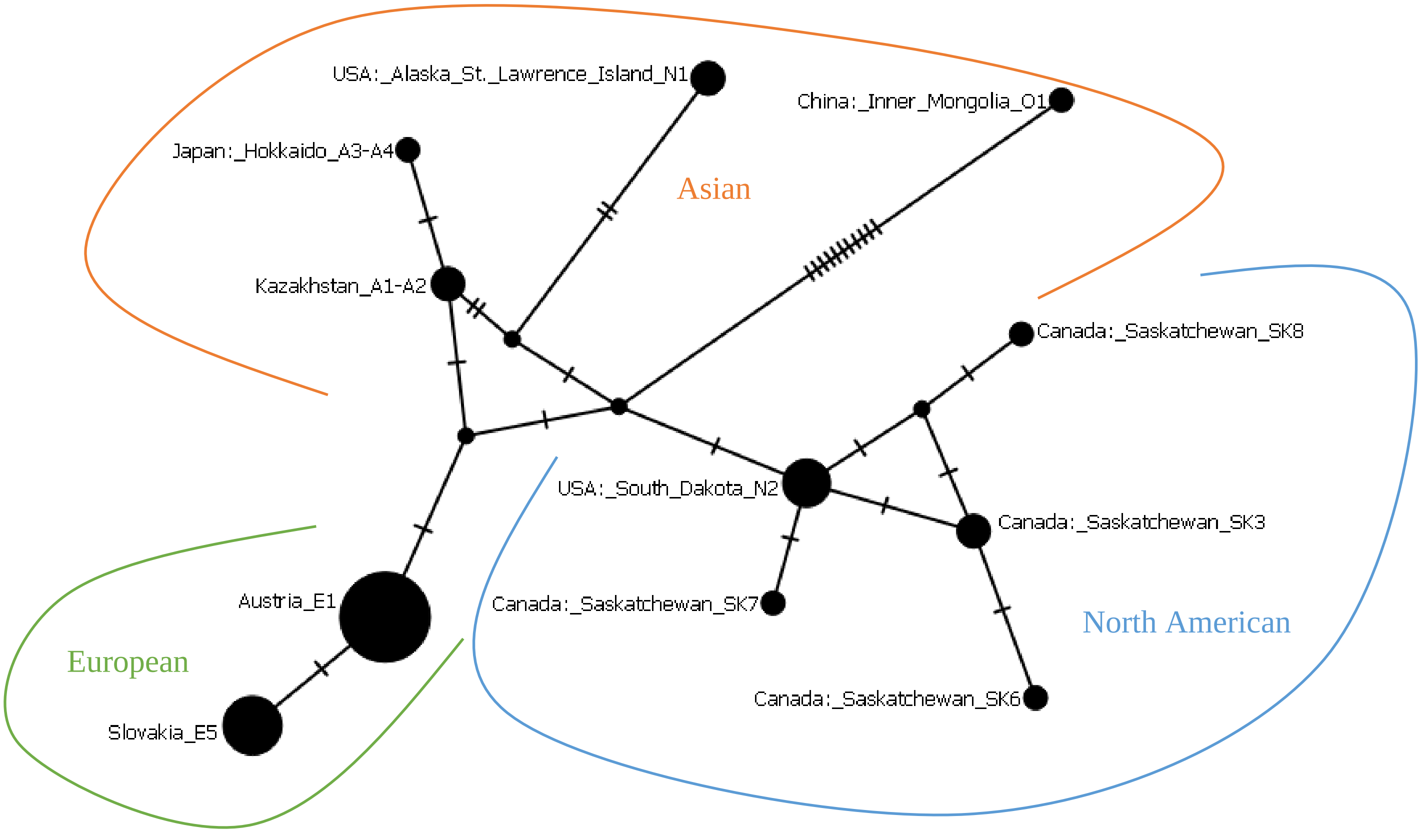


Figure 6 A TCS haplotype network of all reported *E. multilocularis* haplotypes using the *nad2* gene. The GenBank accessions can be found in Table 5.8 in Appendix C.

The number of distinct haplotypes recognized when comparing relationships using the *cob* gene is 10 (Figure 7). Overall, the European haplotypes (Austria-E1, France-E2-E4, and Slovakia-E5) are grouped together with a haplotype from Canada-British Columbia-MC3 being similar. The Asian haplotypes, Kazakhstan-A1-A2 and Kyrgyzstan-A19 are grouped together and have similar relationship with Japan: Hokkaido-A3-A4. The haplotype network shows a distant relationship between the samples found in Alaska (USA: Alaska St. Lawrence Island N1 and USA: Alaska St. Lawrence Island isolate A1-A2) and the United States, which includes North American N2 haplotype (Indiana and South Dakota) and Canada: Saskatchewan SK2, SK6, SK7 and SK8. The four representative sequences from our study (C-KS 52 and 53, C-MO-16 and 42) again being grouped with the Austria_E1 haplotype along with Canada SK1 and Alberta AB1, Poland haplotypes EmPL6 and EmPL10, and domestic dog samples from two case reports in Missouri. All representative sequences for this haplotype network can be found in Table 5.8 in Appendix C.

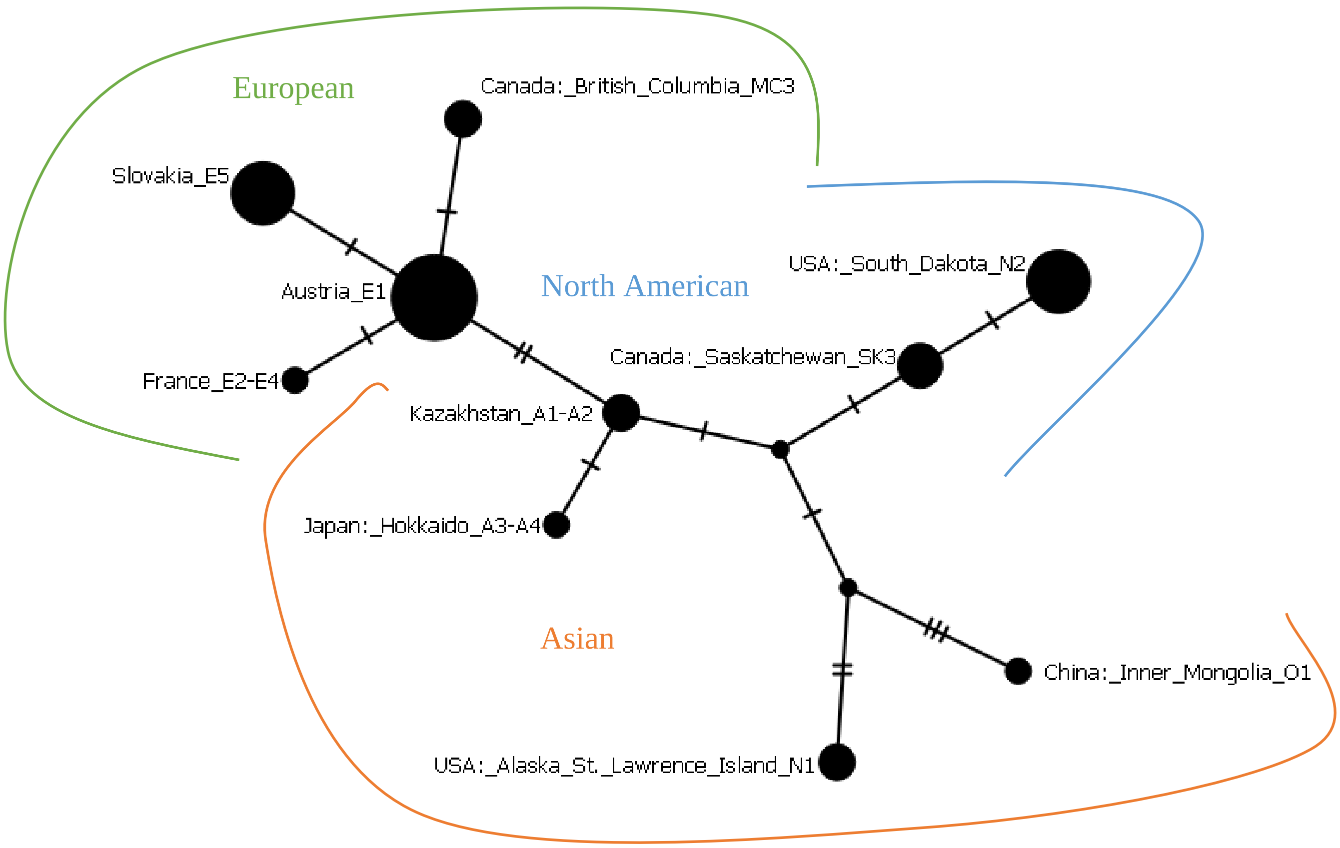


Figure 7 A TCS haplotype network of all reported *E. multilocularis* haplotypes using the *cob* gene.

Morphology

<u>Egg</u>

Eggs produced by *E. multilocularis* are morphologically similar to other taenia-type eggs produced by taeniid tapeworms including *Taenia* spp and *E. granulosus*. Taenia-type eggs are spherical to ellipsoidal and range from 30 – 50 μm in size. The outer shell is passively removed before the egg is freed into the environment. The taenia type eggs have an oncosphere larva, that is immediately infectious, with three pairs of hooks contained in a thick, impermeable embryopore made up of a keratin-like protein (R. C. A. Thompson, 2017).

Metacestode

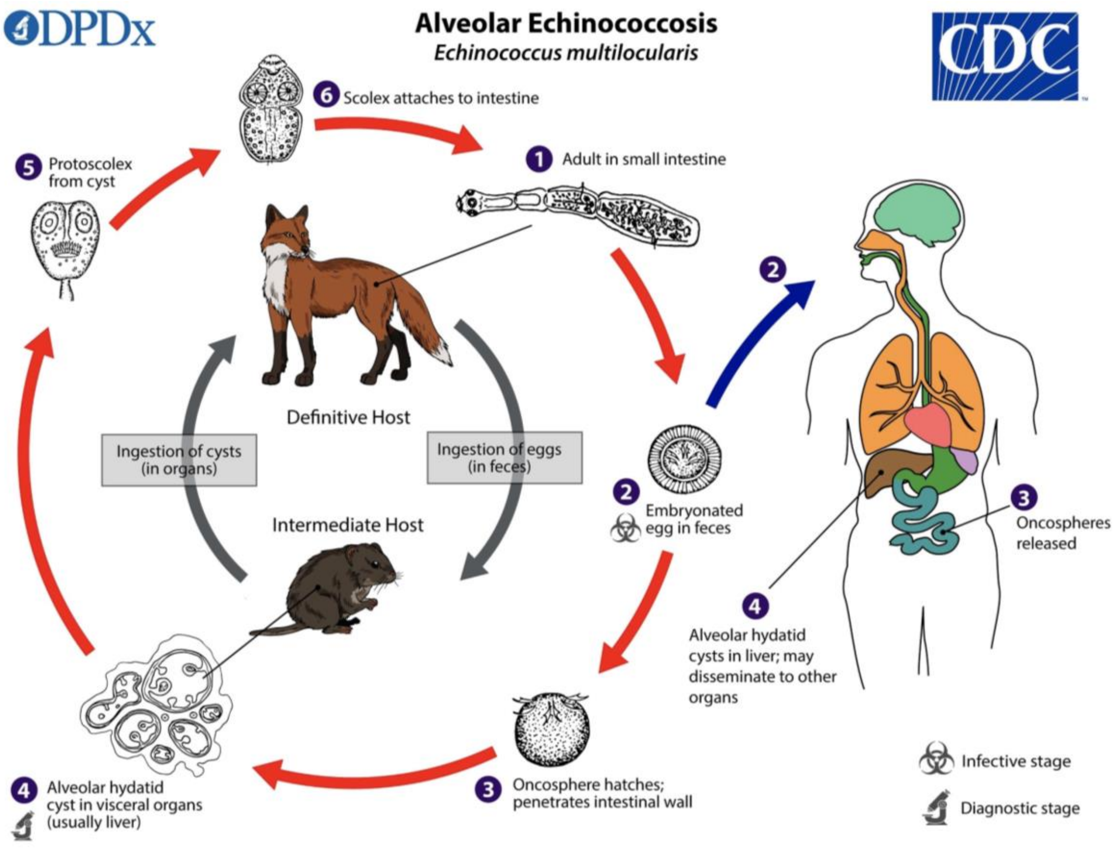
The metacestode stage is a multivesicular, infiltrating mass consisting of numerous small vesicles entrenched in a dense stroma of connective tissue, called a multilocular hydatid cyst (R. C. A. Thompson, 2017). The cysts grow externally by exogenous budding when the germinal layer extends by infiltrating tissue and creating tube-like structures (R. C. A. Thompson, 2017). Then, internally the germinal layer creates brood capsules where protoscolices develop through asexual replication (R. C. A. Thompson, 2017). The cysts take 2 – 4 months to mature in the natural intermediate host, containing a mixture of invaginated protoscolices, brood capsules, and calcareous corpuscles collectively called hydatid sand (R. C. A. Thompson, 2017).

Adult

Adults of *E. multilocularis* are 1.2 – 3.7 mm in length and consists of 3-5 segments. Adults have a scolex with four suckers and an armed rostellum with two rows of hooks. Mature proglottids have one set of reproductive organs including 16-35 testes located laterally to the ovary and uterus The ovary has two branches and a sac-like uterus with no side branches. The genital pore is located in the middle or anterior to the middle of the proglottid (Heidari et al., 2019). Gravid proglottids can have 100 – 1500 eggs per proglottid (R. C. A. Thompson, 2017).

The life cycle, as illustrated in Figure 1.8, begins when adults in the small intestine shed infectious taenia-type eggs in the feces of infected canid hosts. The eggs are ingested by small rodent intermediate hosts, like the meadow vole (*Microtus pennsylvanicus*), southern red-backed vole (*Myodes gapoperi*), and deer mouse (*Peromyscus maniculatus*), and the oncosphere develops into a multilocular hydatid cyst in the liver. Canid definitive hosts predate upon the infected rodent intermediate host and ingests the hydatid cysts containing protoscolices that develop into adults, 28 – 35 days post infection (R. C. A. Thompson, 2017).

Figure 1.8 Life cycle of *Echinococcus multilocularis*. Canid definitive hosts include domestic dogs, red foxes (*Vulpes vulpes*), coyotes (*Canis latrans*) and wolves (*Canis lupus*), while rodent intermediate hosts include voles, lemmings, muskrats, and shrews (CDC, 2019c).



Prevalence

The first report of *E. multilocularis* in Canada was in 1928 in Manitoba and in the United States in the 1960s in North Dakota (E. James & Boyd, 1937; Leiby & Olsen, 1964). Since, then the North American distribution of *Echinococcus multilocularis* has been limited to two endemic geographic regions: i) the Northern Tundra Zone (NTZ) extending along the coast of Alaska, including sub-Arctic islands of St. Lawrence, St. George and Nunivak and the Canadian Arctic and ii) the North Central Region (NCR) including three Canadian provinces (Alberta, Saskatchewan, and Manitoba) and 13 contiguous states in the United States (Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Montana, Nebraska, North Dakota, Ohio, South Dakota, Wisconsin, and Wyoming) (Eckert et al., 2000). Historically in the NTZ region, the Asian haplotypes A2 and A4, and North American haplotype N1 were considered endemic (Nakao et al., 2009a). In the NCR region, the North American haplotype N2 was considered to be endemic (Nakao et al., 2009a).

In the Canadian NCR, the prevalence of *E. multilocularis* in coyotes was 21/92 (25%) in Alberta from 2009-2011, 9/10 (90%) in Saskatchewan from 2010-2012, and 10/43 (23%) in Manitoba in the 1970s (Catalano et al., 2012; K. M. Gesy et al., 2014; Samuel et al., 1978). In the United States NCR, the overall prevalence of *E. multilocularis* in wild canids was ~15% in Indiana, Nebraska, and Ohio, but was reported as high as 20% in South Dakota (Deplazes et al., 2017). Additionally, 18% of wild canids in Illinois tested positive for *E. multilocularis,* while only unpublished reports of positive foxes have been cited in Missouri (Deplazes et al., 2017; Kuroki et al., 2022) (Bates, 1995). Notably, a study in Kansas analyzed 111 coyotes, and none of them were positive for *E. multilocularis* adults (Storandt et al., 2002).

In Canada, the emergence of European strains of *E. multilocularis* started outside the Canadian NCR, in British Columbia, when a domestic dog was diagnosed with Alveolar Echinococcosis (AE) in 2009 (Peregrine et al., 2012). Then wildlife surveillance in British Columbia confirmed establishment of the European strain of *E. multilocularis,* finding 37% (10/27) of coyotes and 17% (1/6) red foxes infected with the tapeworm (K. Gesy, Pawlik, et al., 2013). This started a cascade of reports outside of the known Canadian NCR region, including six AE cases in domestic dogs in Ontario since 2012 (Kotwa et al., 2019). Again, these findings were substantiated when 105/460 (23%) of fecal samples collected from 416 coyotes and 44 foxes were positive for *E. multilocularis* (Kotwa et al., 2019). To date, additional cases of AE in domestic dogs have been reported in the Canadian provinces of Alberta, British Columbia and southern Ontario (Massolo et al., 2019; Peregrine et al., 2012; Skelding et al., 2014). These additional cases come in spite of a study analyzing the prevalence of various parasites in shelter dogs and cats across the Canadian provinces where *E. multilocularis* was not detected in any of the 1,722 fecal samples tested, using centrifugal fecal flotation using Sheather's sugar, from both domestic dogs and cats (Villeneuve et al., 2015a).

In the United States, the first case of a European strain of *E. multilocularis* outside of the historical NCR was AE in a domestic dog in Virginia (A. Zajac et al., 2020). A study collecting feces from red foxes in Virginia showed that 2/296 (0.6%) in Virginia were positive for the European strain of *E. multilocularis* (Polish et al., 2021). Then, the first report of echinococcosis was reported from a 17-week-old puppy in Missouri, which was followed by another case of AE in a 10-year-old Boxer also in Missouri both confirmed as the European strain of *E. multilocularis* (Kuroki et al., 2020, 2022). Areas where domestic dogs are infected with AE suggest that the environmental contamination is high from local predator-prey lifecycles, and those areas should be considered endemic (Kuroki et al., 2022). Wild canid surveillance has resurged in response to the recent case reports of the European strain of *E. multilocularis* in domestic dogs in North America. Recently, 3/48 (7%) coyotes in New York and 2/155 (2%) in Pennsylvania were positive for the European strain of *E. multilocularis* making these the first reports in wild canids and outside of the NCR (Conlon et al., 2023; Garrett, 2021). This is significant because previous research found 22/307 (7%) of coyotes from Quebec, Canada and Maine, USA positive for *E. canadensis* (G8 and G10 cervid strain) not *E. multilocularis* (J. M. Schurer et al., 2018). Additionally, recent wild canid surveillance from Michigan, a confirmed endemic region in the NCR, found only 1/223 (0.4%) of coyotes were infected with *E. multilocularis* (Melotti et al., 2015).

Although cats are not considered suitable definitive hosts, there are two reports of *E. multilocularis* in domestic cats in Saskatchewan and North Dakota (Leiby & Kritsky, 1972; Wobeser, 1971). The study in Saskatchewan found 2% (3/131) of free-roaming cats were positive for *E. multilocularis* with many of them having a low worm burden (30-50 adult worms) with gravid proglottids observed in some infections (Massolo et al., 2014). These infections are considered to be spill-over after an infected house mouse was found in the same area (Massolo et al., 2014). Therefore, domestic cats are not considered suitable hosts and play a marginal role in the life cycle (Romig et al., 2017).

There have been additional reports of intestinal echinococcosis in a grey fox (*Urocyon cinereoaegeneteus*) in Michigan, USA and alveolar echinococcosis in a chipmunk in Southern Ontario, Canada (French et al., 2018; Vande Vusse et al., 1978). Experimental infections in a black bear and a lynx did not produce eggs 31 days post infection, therefore, these species were not thought to be suitable definitive hosts (Massolo et al., 2014). A list of case reports for *E. multilocularis* in wild and domestic canids and humans in the United States, can be found in Appendix A.

Clinical Disease

Echinococcosis occurs when domestic dogs and cats serve as definitive hosts with *E. multilocularis* adults in the small intestine. Typically, intestinal tapeworm infections do not cause overt clinical disease. However, domestic dogs infected with *E. multilocularis* have exhibited a variety of clinical signs reported including, vomiting, diarrhea, hematochezia (bloody diarrhea), weight loss, and inappetence (Evason et al., 2025; Kuroki et al., 2020). In one notable case from Missouri, a 17-week-old puppy presented with hematochezia and was negative on a passive fecal flotation for parasite ova (Kuroki et al., 2020). After, clinical signs returned and radiographs suggested a foreign body, an exploratory surgery was performed with an intestinal biopsy (Kuroki et al., 2020). *Echinococcus multilocularis* adults were discovered by histopathology on an hematoxylin and eosin (H&E) stained slides (Kuroki et al., 2020). Domestic dogs and cats with intestinal echinococcosis are potentially sources of environmental contamination for other domestic animals, humans and the local predator-prey lifecycle (Kuroki et al., 2020). This is troubling because the eggs are immediately infectious and can withstand desiccation in a range of temperatures from -70°C (-110.2°F) to 40°C (104°F) for up to 1 year (R. C. A. Thompson, 2017).

Alveolar Echinococcosis (AE), caused by *E. multilocularis*, occurs when domestic dogs, cats, or humans serve as aberrant intermediate hosts after accidental ingestion of infectious eggs from the environment (Romig et al., 2017). The oncosphere larva hatches and travels mainly to the liver via the bloodstream where the alveolar hydatid cysts begin to grow slowly (Romig, 2017). Alveolar hydatid cysts can seemingly grow undetected for 5 – 10 years within the infected host before the cyst infiltration causes clinical signs (Romig et al., 2017). The hydatid cysts consist of necrotic tissue in the center of the mass, with an inner germinal layer that may or may not have protoscolices, a laminated layer, and a host-induced fibrous layer called the adventitial layer (Kuroki et al., 2022; Williams & Walzthoni, 2023). Clinical signs that have been reported in dogs include painful, distended abdomen, muscle wasting, lethargy, and vomiting (Kuroki et al., 2022; Williams & Walzthoni, 2023). AE can be difficult to treat with prognosis depending on the extent of hydatid cyst growth and infiltration into host tissue. The curative treatment for AE is complete surgical resection of the alveolar hydatid cyst; however, this is often unsuccessful due to the infiltration of cysts into neighboring tissue (Woolsey & Miller, 2021) (Woosley, 2021; Peregrine, 2009). Therefore, a combination of surgery, both complete and partial, and drug therapy using albendazole is recommended (Grüner et al., 2017; Kuroki et al., 2022). If surgery is not an option, the PAIR (percutaneous aspiration injection and reaspiration) method can be used when multiple alveolar hydatid cysts are present that cannot be removed (Williams & Walzthoni, 2023). The largest and most accessible cyst fluid is aspirated, and 95% ethanol is injected into the cyst, allowed to sit for 10-15 minutes, and then reaspirated from the cyst (Williams & Walzthoni, 2023). The PAIR method has varying results with some cysts growing larger and rupturing and others shrinking over time until surgery is possible, or the cysts are undetectable (Williams & Walzthoni, 2023).

Zoonosis

Alveolar echinococcosis (AE), caused by *E. multilocularis*, occurs when humans become aberrant intermediate hosts after accidental ingestion of infectious eggs from the environment through contact with contaminated feces, gardening, or ingestion of contaminated food and water (Frey et al., 2019; Kuroki et al., 2022). In historic autochthonous human AE cases, patients commonly reported a history of fox hunting as a child (Gamble, 1979). The disease process is similar to what occurs when domestic dogs become aberrant intermediate hosts. Hydatid cysts form in distant organs after germinal cells disseminate from cysts and travel via lymph or blood cells to other organs (Kuroki et al., 2022).

Human AE cases are considered highly endemic in portions of Western Europe, Central and Eastern Europe, and a large geographic area spanning from Russia to northern Japan (Polish et al., 2021). The annual incidence of human AE ranges from 0.03 to 1.2 per 100,000 inhabitants, but could be higher in endemic regions (Polish et al., 2021). There are an estimated 18,000 new cases of human AE per year globally with approximately 91% of those cases being reported from China, followed by 42% of cases reported from France, 24% from Germany, and 21% from Switzerland, all areas where cases have dramatically risen (Polish et al., 2021).

The first case reports of Human AE in North America were a man from Winnipeg, Manitoba, Canada and a woman from Minnesota, USA (Gamble, 1979; E. James & Boyd, 1937). In Canada, the drastic increase human AE cases appeared to follow and increase in case reports from wild canids and domestic dogs outside of the Canadian NCR in British Columbia and Ontario. There were 12 human AE cases reported between 2001 and 2014, and an additional six cases reported since 2016 in Alberta (Massolo et al., 2014, 2019). In the United States NCR, outside of the historic case from Minnesota, there are only two human AE cases reported both originating in Vermont (Polish et al., 2021, 2022). The historic human AE case in Minnesota was identical to the North American N2 haplotype, while all new human AE cases in Canada and the United States are all the European strain of *E. multilocularis* (Massolo et al., 2014, 2019; Nakao et al., 2009a; Polish et al., 2021, 2022). The recent case reports of human AE shed more light on the emergence and spread of the European haplotype of *E. multilocularis* outside of the NCR in North America.

Prevention and Control Strategies in Intermediate and Definitive Hosts

The main goal of prevention and control programs are to reduce the risk infection in humans and domestic dogs and to decrease the geographic spread of disease. There have been multiple programs targeting both intermediate and definitive hosts with effective control programs on Ruben Island, Japan and Cyprus (P. S. Craig et al., 2017). Control programs targeted towards rodent intermediate hosts are challenging because rodents can repopulate very quickly and using poison baits can harm large predators (P. S. Craig et al., 2017). Although, there are multiple rodent species that can serve as intermediate hosts, foxes have certain prey preferences with specific rodent species being consumed more than others (P. S. Craig et al., 2017). For example, in Europe, *Arvicola scherman* and the common vole (*Microtus arvalis*), are considered the main intermediate host for *E. multilocularis* (P. S. Craig et al., 2017). In the contiguous United States, the deer mouse (*Peromyscus maniculatus*), the meadow vole (*Microtus pennsylvanicus*) and the house mouse (*Mus musculus*) are the species most commonly found infected with *E. multilocularis* (Deplazes et al., 2017). Therefore, it is recommended to investigate how agricultural and land management maintain rodent populations so that they can be altered in order to control rodent numbers, especially near human settlements (P. S. Craig et al., 2017).

Prevention and control strategies targeting domestic dogs have also been useful, but the effectiveness of these strategies depend heavily upon the role of domestic dogs in maintaining the lifecycle. If domestic dogs serve as the primary definitive host in an endemic region, then this clearly increases the risk of human AE infections. Therefore, regularly treating dogs with praziquantel (PZQ) has been shown to be helpful in not only decreasing the number of infected animals but also decreasing the environmental contamination of eggs. This strategy was successful in St. Lawrence Island, Alaska, where a monthly dog-dosing regimen of PZQ for 10 years decreased the prevalence of *E. multilocularis* from 29% to 1-5% in the vole population, despite there also being an established transmission life cycle between red foxes and rodents (P.

S. Craig et al., 2017). Population management and control has also been attempted in some endemic regions in Asia because stray, unowned, and/or community-owned, free-roaming dogs would significantly contribute to the zoonotic risk and transmission to humans. However, these programs are often unsuccessful because culling domestic dogs is often seen as unethical and is widely opposed in many communities (P. S. Craig et al., 2017).

Prevention and control strategies targeting wild canids, such as red foxes, have also been used with mixed success. In European countries, fox populations significantly increased when rabies was eradicated, and this was exacerbated by continual decrease in fox hunting and trapping (Romig et al., 2017; Schweiger et al., 2007). Any hunting efforts to decrease population have been countered by the fox's ability to successfully reproduce and rebuild the population. Culling fox populations was successful in the eradication of *E. multilocularis* on Ruben Island in Japan. Red foxes were considered an invasive species after 12 pairs were introduced between 1924 and 1926 for rodent control (P. S. Craig et al., 2017). The control program, 1950 to 1970, helped to eradicate foxes on the island and capture and necropsy domestic dogs and cats. This aggressive, yet effective, program helped decrease the number of human AE cases from 111 in 1964 to no new cases reported since 1994 (P. S. Craig et al., 2017). Lastly, the use of PZQ baits can be challenging, and costly, because baits must be administered on a monthly basis to account for reinfection. However, these programs have been utilized successfully in foxes in countries like Germany, Switzerland, Japan, Slovak Republic and France (Romig et al., 2017).

1.3 Current and Historical Diagnosis of Tapeworms of Veterinary Importance

1.3.1 Historical Diagnosis

1.3.1.1 Passive (or benchtop) fecal flotation

The goal of performing a fecal flotation is to concentrate parasite eggs while simultaneously separating them from fecal debris (A. M. Zajac et al., 2021). A passive fecal flotation is typically used in veterinary clinics where centrifugation is not available. There are limitations when performing a passive fecal flotation to detect tapeworm eggs. First, tapeworm eggs are dense and heavy, taenia-type eggs have a SPG of 1.2-1.3 and *Dipylidium* egg packets have a SPG of 1.30-1.45. Therefore, a flotation solution with an SPG of at least 1.27 is needed in order to float and recover eggs (David & Lindquist, 1982; Dryden et al., 2005). Sheather's sugar flotation solution (SPG 1.27), which is the preferred flotation solution used to float most parasite eggs, has a low sensitivity in passive flotations because the viscosity doesn't allow eggs to passively float (Dryden et al., 2005). Passive fecal flotations using a salt flotation solution (Sp. G < 1.18) are unable to float eggs from *Taenia* spp. and *Dipylidium* and can quickly crystalize on the microscope slide, causing a distortion of parasite eggs making them difficult to identify (A. M. Zajac et al., 2021). Therefore, tapeworm infections often go undiagnosed when using this method, as was the case of echinococcosis in a 17-week old puppy in Missouri, USA (Kuroki et al., 2020).

1.3.1.2 Adult worm identification

Historically, tapeworm species have been described using the similarity concept, which is centered primarily on morphological differences or host identity, to identify different tapeworm species (Padgett et al., 2005). Tapeworm proglottids are normally shed in individual, or multiple, segments, stained and preserved to identify key morphological features such as tegument, genital pores, reproductive structures, and eggs.

This method, while useful for identifying tapeworm morphology, has brought widespread confusion when it comes to identifying tapeworm species. These were the hurdles that plagued

Kuchta & Scholz (2017) when aiming to understand the epidemiology and distribution of broad tapeworms (Kuchta & Scholz, 2017). Just relying on morphology alone did not account for the high intraspecific, host-related variability and uniformity of general morphological features within a tapeworm taxon (Kuchta et al., 2024). For example, Anderson (1972) observed morphologically smaller mature segments, with smaller ovaries and uteri, of *Diphyllobothrium dendriticum* in golden hamsters (*Mesocricetus auratus*) infected with single tapeworm infections versus heavy infections (Andersen, 1972). Meanwhile, also observed no correlation with increasing population size when looking at differences in scolex height and length, neck length, number of segment anterior to the primordia or number of segments anterior to the first gravid uterus (Andersen, 1972). These observations lead to a large number of species identified from the same hosts and regions bringing to question the validity of the taxa (Kuchta & Scholz, 2017). Additionally, it is common to find reports of new species from ill-preserved specimens that lack well-defined morphological detail (Kuchta et al., 2024). These difficulties were also observed in *Mesocestoides* spp. and *Dipylidium caninum* (J. R. J. Jesudoss Chelladurai & Brewer, 2021)

1.3.1.3 Arecoline Purgation

Arecoline was used as a crude dewormer and pre-mortem diagnostic test for *Echinococcus* spp. infections in domestic dogs (Siles-Lucas et al., 2017). Arecoline hydrobromide is a synthetic compound given orally, or rectally, that relaxes the smooth muscles of the intestine and paralyzes worms (P. S. Craig et al., 2017). Arecoline was used in the late 19th to early 20th centuries including the 1960s as a surveillance method for rural-owned domestic dogs in Iceland, New Zealand, Tasmania and Cyprus (P. S. Craig et al., 2017).

Limitations to arecoline usage is that some domestic dogs may take >60 minutes to purge, may need a second dose, or may not purge at all. This method has a sensitivity of 68%

because the tapeworms recovered have to be preserved and identified, and a specificity of 100% because if worms are not present in the gastrointestinal tract, then they will not be recovered (Deplazes et al., 1999; Eckert, 2003; Eckert et al., 2001). The method is also laborious and time consuming in large-scale surveillance (P. S. Craig et al., 2017). Arecoline can cause intestinal perforation, which can lead to death, and is contraindicated in younger, older, and pregnant dogs (Eckert et al., 2001). Despite still being used today, this method is dangerous and potentially unreliable.

1.3.1.4 Sedimentation and counting technique (SCT)

Sedimentation and counting technique (SCT) was once considered the ‘gold standard’ for post-mortem diagnosis of *Echinococcus multilocularis* in domestic and wild canids. The process involves collecting intestines at necropsy, scraping them to remove the intestinal lining and counting to evaluate infection intensity (Conraths & Deplazes, 2015). It was recommended for the use of large-scale wild canid surveillance.

Limitations for SCT include being labor-intensive, time consuming, requires special equipment, and the skillset to morphologically identify *Echinococcus* spp. tapeworms (K. Gesy, Pawlik, et al., 2013). SCT requires sedimentation of sample that may take hours before sifting can begin and can still lead to a false negative if there is a low infection intensity (Conboy, 2009) (Thompson, 1986; Conboy, 2009). This method also uses saline, which can distort worm structure making it difficult to identify morphologically and molecularly (K. Gesy, Pawlik, et al., 2013).

1.3.2 Current Diagnostic Techniques

1.3.2.1 Centrifugation Fecal Flotation

Centrifugal fecal flotation is the most common fecal diagnostic technique used to identify parasite eggs and diagnose tapeworm infections (A. M. Zajac et al., 2021). Centrifugal fecal flotation requires two to five grams of fresh feces mixed with Sheather's sugar (SPG 1.27-1.33) most commonly, but Zinc Sulfate (SPG 1.24) can also be used, flotation solution and centrifuged to float the egg stages. Limitations for this method include low sensitivity of recovering tapeworm eggs because the eggs are often contained in proglottids that are shed in the feces (Little et al., 2023). Breaking up the proglottids during feces homogenization would help recovery, but proglottids are shed intermittently and may not be found in every fecal sample (Conboy, 2009; Dryden et al., 2005; A. M. Zajac et al., 2021). Even when using a high specific gravity solution, such as Sheather's sugar, cestode eggs such as taenia-type eggs (SPG 1.2 – 1.3) and *D. caninum* egg packets (SPG 1.30-1.45) are dense and heavy and may not float (Dryden et al., 2005; Little et al., 2023). Additionally, taenia-type eggs cannot be identified past the family level because all taeniid eggs are morphologically similar (Urqhuart et al., 1996). Lastly, this method requires experienced personnel that can correctly identify and distinguish parasite eggs (Dryden et al., 2005).

1.3.2.2 Molecular detection of DNA in feces

Molecular detection using polymerase chain reaction (PCR) can be used to detect pre-patent and current tapeworm infections. This has become a widely accepted method, not only for taxonomic classification, but is especially useful for adult and/or egg samples are damaged or degraded. Perianal swabs have been used for the detection of *D. caninum* infection. Perianal swabs collect remnant parasite material from proglottids concentrated in the perianal region (Elsemore et al., 2023; Labuschagne et al., 2018). However, perianal swabs cannot be used to determine active infection because residual parasite material can persist after successful treatment (Labuschagne et al., 2018; Muchaamba et al., 2021). A fecal PCR for *D. caninum* has also been used, but was shown to underperform when compared to PCR of the perianal swab (Elsemore et al., 2023). The underperformance of the *D. caninum* fecal PCR to perianal swab PCR could be due to fecal inhibitors affecting sensitivity of fecal PCR and the intermittent shedding of proglottids as well as egg packets not being evenly distributed in the sample (Monteiro et al., 1997; G.-Q. Zhu et al., 2019). Diagnostic companies have used molecular detection for the rapid screening of feces from domestic dogs and cats for multiple parasites at once. Specifically, Antech Diagnostics Inc. (Fountain Valley, CA) has created a multiplex PCR that detects over 20 common parasites infecting domestic dogs and cats including tapeworms (Evason et al., 2025; Leutenegger et al., 2023). The KeyScreen GI Parasite PCR (Antech Diagnostics Inc.) is a copro-PCR that amplifies DNA from the following tapeworms: *D. caninum*, *Taenia* spp., *E. granulosus*, and *E. multilocularis.* Recently, the KeyScreen GI Parasite PCR (Antech Diagnostics Inc.) was able to detect 26 of 2,333,797 dog fecal samples from a reference laboratory as *E. multilocularis* in multiple states and provinces in North America (Evason et al., 2025; Peregrine et al., 2012). In a research setting, a multiplex PCR was developed to differentiate *Taenia* spp., *E. granulosus*, and *E. multilocularis* for the screening of wild canid feces and molecular identification of taenia-type eggs in those samples (Trachsel et al., 2007).

1.3.2.3 Coproantigen testing

Immunologic tests have been used to detect host antibodies to certain tapeworm species but have a problem with differentiating exposure from active infection (D. J. Jenkins et al., 1990). This is worrisome, especially when detecting *Echinococcus* spp. infections, because it is difficult to start treatment and control programs in endemic areas. A coproantigen ELISA was created for *E. multilocularis* with the capability of detecting antigen from eggs and adults and the ability to detect pre-patent infections (Allan et al., 1992; Deplazes et al., 1992). This test still comes with limitations, struggling to detect low worm burden and cross-reactivity with *Taenia* spp., which lead to decreased diagnostic sensitivity and specificity, respectively (Torgerson & Deplazes, 2009). In *D. caninum,* however, a coproantigen immunoassay created and used by IDEXX diagnostic laboratory (IDEXX Laboratories, Inc., Westbrook, ME) has increased diagnostic sensitivity able to detect infections as early as 10 days before proglottid shedding in some cases (Elsemore et al., 2023). In a recent study evaluating the fecal samples from pet-owned and shelter dogs in six states in the United States, the coproantigen immunoassay increased detection of *D. caninum* by several fold (Little et al., 2023). A total of 877 dogs (589 pet and 288 from municipal shelters) were tested using a zinc sulfate centrifugal flotation, *D. caninum* coproantigen, and perianal swab PCR. In pet-owned dogs, *D. caninum* infection was detected in 2.2% of samples by coproantigen, 0% by fecal flotation and 1.2% by perianal swabs and in shelter dogs *D. caninum* infection was detected in 12.5% of samples using coproantigen, 0.3% using fecal flotation and 11.1% using perianal swabs (Little et al., 2023).

1.3.2.4 Scraping, filtration and counting technique (SFCT)

Sedimentation, filtration, and counting technique (SFCT) is an improved method of the SCT and considered the new 'gold standard' for large-scale surveillance of *Echinococcus* spp. tapeworms in wild canids. This process involves freezing intestines at -80ºC for at least 7 days for biosafety, thawing the samples, cutting the intestines into sections, and then longitudinally cutting the intestines open to access intestinal lining (Duscher et al., 2005; Eckert & Deplazes, 2004). These samples are shaken in jars of distilled water (dH_2O), then scraped between two fingers to remove any remaining lining. The slurry is then sieved once through a large mesh sieve (1 mm pore size) to remove large debris and macroscopic parasites and again through a smaller sieve (150 um pore size) to retain all scolices and segments (K. Gesy, Pawlik, et al., 2013). The addition of the filtration step helped to improve the sensitivity of *Echinococcus* sp. recovery in one study from 25% (21/85) using SCT to 27% (23/85) using the SFCT (K. Gesy, Pawlik, et al., 2013). Also helped to reduce false positive and negative results by decreasing intestinal villi that could be mistaken for tapeworms (K. Gesy, Pawlik, et al., 2013).

Limitations still exist for this study, despite being improved from the SCT. This method is still time consuming despite decreasing the average processing time by 68.5 minutes per sample (K. Gesy, Pawlik, et al., 2013). This method requires both space to process intestines and a skillset to morphologically identify *Echinococcus* spp. adults.

1.4 Treatment and Prevention of Tapeworms of Veterinary Importance

1.4.1 Treatment with anthelmintics

Treatment of tapeworms of veterinary importance is predominantly achieved by using two drugs: praziquantel and epsiprantel (Bowman, 2021). Belonging to the isoquinolone drug class, these drugs are safe, effective and approved anthelmintics for use in the United States (Bowman, 2021). Treatment can also be achieved using other anthelminthics such as Fenbendazole and

1.4.1.1 Praziquantel

Praziquantel (PZQ) is an isoquinolone primarily used for the treatment of tapeworms in domestic dogs and cats (Bowman, 2021). The biochemical mechanism of action of praziquantel is unknown in tapeworms (C. M. Thomas & Timson, 2020). However, studies evaluating the effect of praziquantel on the fluke *Schistosoma mansoni*, have shown that praziquantel acts on calcium channels and causes rapid release of calcium into the cytoplasm of cells, leading to muscle contraction and paralysis (Coles, 1979). The paralysis prevents mating and egg production and enables expulsion of adult parasites by the host immune system (Coles, 1979). Praziquantel also caused vacuolization of the tegument by slowing depolarization leading to regionalized lesions, erosion of the tegument, exposure of worm antigens important for immune function and eventually expulsion of worms from the host (Park & Marchant, 2020).

Praziquantel has marked efficacy against a wide range of tapeworms. When administered orally, PZQ has complete absorption and rapid distribution throughout the body and across the blood-brain barrier (Bowman, 2021). Praziquantel is metabolized to 4'-hydroxy-praziquantel in dogs, inactivated in the liver, and 80% of the drug is eliminated in the urine (Bowman, 2021; Dayan, 2003). Praziquantel is very safe with only vomiting recorded at dosages greater than 200 mg/kg; therefore, there is no establish oral LD50 for domestic dogs (Bowman, 2021; Dayan, 2003).

Praziquantel is labeled for the routine treatment of many common tapeworms infecting domestic dogs and cats including *Dipylidium caninum, Echinococcus granulosus, E. multilocularis, Mesocestoides* spp., *Taenia taeniaformis, T. pisiformis*, and *T. hydatigena* (Bowman, 2021). PZQ can be administered orally or injected subcutaneously (SQ) at 5 mg/kg to treat the aforementioned tapeworm species (Bowman, 2021). The false tapeworms, *D. latus* and *Spirometra* sp. 2, can be more difficult to treat so it is recommended to increase the dose to 25 mg/kg for two consecutive days (Bowman, 2021).

1.4.1.2 Epsiprantel

Epsiprantel, another cestocidal isoquinolone, has poor oral bioavailability, no known metabolites, and only 0.1% of the drug is recovered in the urine (Riviere & Papich, 2018). Epsiprantel is chemically related to praziquantel and has similar affects calcium homeostasis within the parasite by damaging to tegument and making the parasite vulnerable to lysis and digestion in the host gut (Riviere & Papich, 2018). Epsiprantel has low gastrointestinal absorption, therefore it is a safe drug. However, it is unknown if epsiprantel is safe to give in pregnant dogs and cats (Bowman, 2021). The LD50 oral dose in mice and rats was greater than 5000 mg/kg. In dogs, doses 36 times the labeled dose were well tolerated. Doses given 40 times the therapeutic dose caused vomiting in some kittens (Riviere & Papich, 2018).

Epsiprantel is labeled for the removal of common tapeworms in domestic dogs and cats such as *Dipylidium caninum*, *Taenia hydatigena*, *T. pisiformis*, and *T. taeniaformis* (Bowman, 2021). It is available in an oral tablet at 2.75 mg/kg for cats and 5.5 mg/kg for dogs.

1.4.1.3 Fenbendazole

Fenbendazole belongs to the benzimidazole drug group that have a broad spectrum of anthelminthic activity. This drug group is overall remarkably safe. Fenbendazole acts by binding to the parasite β-tubulin causing disruption of cell division, cell shape maintenance, cell motility, cell secretion, nutrient absorption and intracellular transport (Riviere & Papich, 2018). The loss in cell function leads to decreased glucose uptake and loss of transport of secretory vesicles and ultimately leads to death of the parasite (Riviere & Papich, 2018). Fenbendazole has poor water solubility so dosages are given by mouth (Riviere & Papich, 2018). Despite this, fenbendazole is lipophilic and remain in the bloodstream for longer periods of time (Riviere & Papich, 2018). Fenbendazole can be used to treat *Taenia* spp. tapeworms, except for *E. granulosus* and *E. multilocularis*, at 50 mg/kg daily for 3 consecutive days (Riviere & Papich, 2018).

1.4.1.4 Nitroscanate

Nitroscanate (4-(4’-nitrophenoxyl) phenyl isothiocyanate) is a synthetic chemotherapeutic agent with broad spectrum activity against tapeworms of dogs (Bowman et al., 1991). The mechanism of action of nitroscanate in tapeworms is unknown (Bowman et al., 1991). In other flatworms, like flukes, nitroscanate has been shown to inhibit the uptake of adenosine triphosphate, which inhibits cellular processes like muscle contraction, the transport of ions across the cell membrane, and energy for rebuilding and repairing cellular components (Cornish & Bryant, 1976). Field trials evaluating the efficacy of nitroscanate in dogs with *Taenia hydatigena* and *Echinococcus granulosus* using a particle size of 10-20 μm showed efficacy in clearing both infections (Gemmell & Oudemans, 1975). However, the medication given at this dose caused vomiting, diarrhea, and a transient tranquilizing in some dogs (Gemmell & Oudemans, 1975). So, the active ingredient of nitroscanate was micronized to a particle size of 2-3 μm and used in a field trial for the elimination of *E. granulosus* and *T. pisiformis* in domestic dogs and showed reduced worm burdens without the increase in toxic effects (Gemmel et al., 1977). Nitroscanate has also shown efficacy against *Dipylidium caninum* with 100 percent clearance of worms at 200 mg/kg and 97.9 percent at the micronized dose of 50 mg/kg (Richards & Somerville, 1980). Nitroscanate is not approved by the Food & Drug Administration (FDA) and is not available in the United States.

1.4.1.5 Nitazoxanide

Nitazoxanide is a nitrothiazolyl-salicylamide, a broad spectrum antiparasitic drug used for the treatment of protozoans (i.e. *Cryptosporidium* sp. And *Giardia* sp.) (De Lima et al., 2021). Nitazoxanide acts on the glutamate-gated ion channel. Several studies have shown that nitazoxanide affects the cystolic and mitochondrial energetic metabolism of *Taenia crassiceps* (De Lima et al., 2021). Additionally, nitazoxanide also effects the parasite morphology by inducing lesions on the mitochondrial membrane (De Lima et al., 2021). Nitazoxanide also affects hydatid cysts by attacking the germinal layer to prevent growth and production of protoscolices (De Lima et al., 2021). Nitazoxanide is FDA-approved for the treatment of Giardiasis in humans (White Jr, 2004). In dogs, nitazoxanide has been effective for extra-label use for the treatment of tapeworms (C. Liu et al., 2015; Shakya et al., 2018).

1.4.2 Prevention using broad spectrum products containing praziquantel

Broad spectrum products have different combinations, dosing regiments, and applications to fit pet and owner preferences and lifestyles. There are a few approved broad-spectrum products available for the prevention of common tapeworms in domestic dogs and cats including *Dipylidium caninum*, *Taenia pisiformis*, *T. taeniaformis*, and the adult stages of *Echinococcus granulosus* and *E. multilocularis* in the United States (see Tables 1.3 and 1.4).

Table 1.3 Canine broad spectrum products effective against tapeworms in the US

Product Name	Min. weight (lbs)	Min. age (weeks)	Drugs (minimum dose)	Route of Application	Parasites Treated
Credilio Quattro	3.3	8w	Lotilaner (20 mg/kg) + moxidectin (0.02 mg/kg) + praziquantel (5 mg/kg) + pyrantel (5 mg/kg)	PO – tablet	Dc, Tp, Eg, Di, Tc, Tl, Us, Cf, Aa, Dv, Is, Rs
Interceptor Plus	2	6w	Milbemycin oxime (0.5 mg/kg) + praziquantel (5 mg/kg)	PO – monthly chew	Tp, Em, Eg, Di, Ac, Tc, Tl, Tv
Iverheart Max		8w	Ivermectin (0.006 mg/kg) + pyrantel (5 mg/kg) + praziquantel (5 mg/kg)	PO – monthly chew	Dc, Tp, Di, Ac, Ab, Us, Tc, Tl
Sentinel Spectrum	2	6w	Milbemycin oxime (0.5 mg/kg) + lufenuron (10 mg/kg) + praziquantel (5 mg/kg)	PO – monthly chew	Tp, *Em*, Eg, Di, Ac, Tc, Tl, Tv

Dc = *Dipylidium caninum*; Tp = *Taenia pisiformis*; *Em* = *Echinococcus multilocularis*; Eg = *E. granulosus*; Di = *Dirofilaria immitis*; Ac = *Ancylostoma caninum*; Ab = *Ancylostoma bazilense*; Us = *Uncinaria stenocephalus*; Tc = *Toxocara canis*; Tl = *Toxascaris lenonina*; Tv = *Trichuris vulpis*; Cf = *Ctenocephalides felis*; Aa = *Amblyomma americana*; Dv = *Dermacentor variabilis*; Is = *Ixodes scapularis*; Rs = *Rhipicephalus sanguineus*

Table 1.4 Feline broad spectrum products effective against tapeworms in the US

Product Name	Min. weight (lbs)	Min. age (weeks)	Drugs (minimum dose)	Route of Application	Parasites Treated
NexGaurd Combo	1.8	8	Esafoxolaner (1.44 mg/kg) + eprinomectin (0.48 mg/kg) + praziquantel (9.98 mg/kg)	Spot-on	Dc, Di, At, Ab, Tc, Aa, Is
Profender	2.2	8w	Emodepside (3 mg/kg) + praziquantel (12mg/kg)	Spot-on	Dc, Tt, At, Tc

Dc = *Dipylidium caninum*; Tt = *Taenia taeniaformis*; Di = *Dirofilaria immitis*; At = *Ancylostoma tubaeformae*, Ab = *Ancylostoma brazilense*; Tc = *Toxocara cati*; Aa = *Amblyomma americana*; Is = *Ixodes scapularis*

1.4.3 Praziquantel Resistance

Resistance has been widely documented in veterinary parasitology, most notably in *Dirofilaria immitis* (canine heartworm) and *Ancylostoma caninum* (canine hookworm) (Bourguinat et al., 2015; Jimenez Castro et al., 2023; Venkatesan et al., 2023). Resistance is described as the phenomenon when heritable genetic changes in a population of helminths result in a larger proportion of the population remaining alive following the administration of a previously effective drug dose (Loftus et al., 2022).

Recently, the first documented case of PZQ resistance in *D. caninum* was reported in five domestic dogs from Michigan, Colorado, Minnesota, and two dogs from Iowa (J. Jesudoss Chelladurai et al., 2018). None of the cases could be resolved with labeled doses of PZQ, Epsiprantel, or higher doses and durations (J. Jesudoss Chelladurai et al., 2018). Although one case was lost to follow-up, the other cases resolved eventually using a variety of treatment options (J. Jesudoss Chelladurai et al., 2018). One case was successfully treated with nitroscanate, and two cases were treated with a combination of pyrantel/praziquantel/oxantel compound, where pyrantel was thought to play a synergistic role with the other compounded anthelmintics (J. Jesudoss Chelladurai et al., 2018). One case ceased shedding proglottids spontaneously after several months of treatment and it was proposed that the worms reached their natural lifespan and were expelled from the body (J. Jesudoss Chelladurai et al., 2018). Another case of a probable PZQ-resistant case of *D. caninum* was successfully treated with nitroscanate in New York state (Loftus et al., 2022).

The first international report of PZQ-resistance was reported from Europe (Oehm et al., 2024). Treatment was ultimately successful with mebendazole at an increased dose of 86.2 mg/kg after treatments with PZQ, fenbendazole, epsiprantel, a combination of praziquantel/pyrantel/febantel, and lower doses of mebendazole were unsuccessful (Oehm et al., 2024).

Anecdotal cases of PZQ resistance are received monthly to the Parasitology Department at Kansas State University Veterinary Diagnostic Lab and are presumably increasing in clinical practice. In order to ensure success in treating potentially PZQ-resistant cases it is key to ensure correct identification of the tapeworm species, there is adequate flea control, correct administration of anthelminthics and broad-spectrum products and, most importantly, owner compliance (Oehm et al., 2024).

Chapter 2 - Prevalence *of Dipylidium caninum* in *Ctenocephalides felis* from cats, dogs, and homes in Tampa, FL

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2.1 Abstract

Background: The cat flea, *Ctenocephalides felis,* is a common external parasite that can transmit a variety of pathogens, including *Dipylidium caninum,* a zoonotic tapeworm that infects the small intestine of cats and dogs.

Methods: For this study, fleas were collected from cats, dogs, and the home environment of residential homes in the Tampa, FL area. A total of 1391 fleas were collected: 281 fleas from 40 cats, 99 fleas from 8 dogs, and 1011 fleas from 24 home environments. A PCR targeting 28s subunit was utilized to detect *D. caninum* within individual and pools of cat fleas, and sequences were compared to known canine and feline *D. caninum* genotypes.

Results: A total of 213 pools of three fleas and 65 individual fleas were tested, and of those, 74 pools were from cats, 26 pools were from dogs, and 113 pools were from the environment. Of the pools tested, 8/213 (3.8%) total pools were positive for *D. caninum;* 2/74 cat pools (2.7%); 0/26 dog pools (0.0%); 6/113 environmental pools (5.3%). A total of 4/65 (6.2%) of individual fleas from the environment were also positive for *D. caninum*. There was no significant difference in the prevalence of *D. caninum* between the three pool groups ($X^2 < 1.45$, $p > 0.22$) nor between the pools and individual fleas tested ($X^2 = 0.69$, $p = 0.405$). All *D. caninum* sequences shared 100% identity with published feline genotypes of the tapeworm.

Conclusions: Given there was no significant difference in the percentage of infected on-animal or environmental fleas, the tapeworm is likely found at a low level throughout all cat flea populations. Additionally, pooled flea testing may be a valid method estimating infection rate for more widespread surveillance efforts. These findings provide further evidence of the importance for the use of consistent and effective flea control as a method for tapeworm prevention for cats and dogs.

2.2 Introduction

Dipylidium caninum, the flea tapeworm, is the most common tapeworm of domestic dogs and cats in North America. The most common vector for *D. caninum* is the cat flea, *Ctenocephalides felis*. The cat flea is the most common and important ectoparasite found on domestic cats and dogs worldwide (Rust, 2017). Infection with *D. caninum* occurs when domestic cats, dogs, or rarely humans ingest fleas infected with the cysticercoid larval stage. Adults develop, mature, and live in the small intestines and shed proglottids containing eggs packets in the feces. In order to prevent new and recurrent infections of *D. caninum*, house pets should be treated for current infections and placed on monthly broad-spectrum preventative that includes flea control to prevent reinfection.

Recent publications have documented molecular differences in *D. caninum* specimen that suggest there are likely two separate species, one which predominantly infects cats and another that infects dogs (J. R. J. Jesudoss Chelladurai et al., 2023; Labuschagne et al., 2018; Low et al., 2017). These molecular differences have been confirmed with infection studies where cats and dogs were infected with both genotypes (Beugnet et al., 2018). *Dipylidium caninum* were more likely to successfully infect their corresponding host, and those tapeworm genotypes in the correct host also had a shorter prepatent period and a longer lifespan (Beugnet et al., 2018). All of this suggests that, while both cats and dogs can be infected with either genotype, the genotypes have host-adapted to more successfully live in either cats or dogs, respectively. In addition, there have been several case reports documenting *D. caninum* that was not susceptible to normal, and even elevated, doses of praziquantel (J. R. J. Jesudoss Chelladurai et al., 2023; Loftus et al., 2022; Oehm et al., 2024). All of the reported cases were in dogs, but the publications do not specify the genotype of the *Dipylidium* collected.

Despite *D. caninum* being the most common tapeworm of dogs and cats worldwide, being zoonotic, and exhibiting newly documented drug-resistant phenotypes, there is a lack of information about this tapeworm within the flea intermediate host. Specifically, very few publications describe the percent of fleas infected with *D. caninum* in a given area. In fact, to the author's knowledge, there are no studies documenting the percent of fleas that are positive for *D. caninum* in North America. Worldwide, there are several studies that report 1.5 – 5.2% of cat fleas tested were positive for *D. caninum*, although a variety of pooling and testing methods have been utilized (Abdullah et al., 2019; Beugnet et al., 2014; Low et al., 2017). Additionally, previous research suggests there may also be differences in the percentage of positive fleas removed from cats compared to dogs (Beugnet et al., 2014). This study aims to provide a first description of the percent of fleas infected with *D. caninum* in North America as well as assess any differences in infection percentages between fleas removed from cats, dogs, or collected in the home environment.

2.3 Methods

2.3.1 Ethical approval

All animal handling and treatment procedures were reviewed and approved by Kansas State University IACUC #4704.

2.3.2 Enrollment

This study is a portion of a larger study evaluating the efficacy of lotilaner (Credelio®CAT; Elanco Animal Health) in controlling natural flea infestations of cats in residential homes in the Tampa, FL area. To be included in the "efficacy study", cats had to be healthy and spend the majority of time indoors, no on-animal or premises treatments could have been recently given, and the owner had to consent to in-home evaluations for the 12-week study period. Full enrollment and inclusion criteria can be found in the publication for the efficacy portion of this study (Sutherland et al., In Review).

2.3.3 Flea collections

Fleas were counted on each cat enrolled in the study using a flea combing technique described in a previous study (Dryden et al., 1994). Cats or dogs with ≥ 10 fleas had a subset removed for testing and the remainder were returned to the animal to evaluate product efficacy. Animals with 10 – 14 fleas, three were removed; 15 – 24 fleas, six removed; 25 – 49 fleas, nine removed; ≥50 fleas, 12 fleas removed.

The environmental flea population in each participating home was assessed using validated intermittent light traps (Dryden & Broce, 1993). Two traps were placed in separate rooms for a 16 – 24 h period. The trap locations were selected based on owners' observations of where animals spent most of their time. Fleas were left on the adhesive traps at -20 ^{o}C until they could be processed in the laboratory.

All fleas utilized in this study were collected on Day 0 prior to any drug treatments. The fleas were morphologically identified as *Ctenocephalides felis* by a board-certified veterinary parasitologist. Fleas were divided into pools of three based on household and source (cat, dog, trap), and each individual pet or trap could have up to four pools of three fleas. All additional fleas were retained at -20^{o}C for individual testing.

2.3.4 DNA extraction and PCR

Pools of fleas were placed in 500µl of 95% EtOH and vortexed for 30s. The EtOH was then removed, and the step was repeated using nuclease-free water (Sridhar et al., 2022). The fleas were then removed and placed into ZR BashingBead™ Lysis Tubes (0.1- & 2.0-mm Beads) (Zymo Research; Irvine, CA). The pools were homogenized for 1 minute using a

FastPrep-24™ Classic bead beating grinder and lysis system (MP Biomedicals; Santa Ana, CA) and DNA extracted using the QIAwave DNA Blood & Tissue Kit (Qiagen; Hilden, Germany). A conventional PCR targeting the 28S rDNA was performed using primers (DC28S-1F and DC28S-1R) and protocol previously described by Beugnet et al., (2014). DNA from positive samples was purified using Wizard DNA Cleanup System (Promega; Madison, WI) and set for Sanger sequencing at Eurofins Scientific (Lancaster, PA). Sequences were compared with known *D. caninum* strains on GenBank.

2.4 Results

A total of 1391 fleas from 47 homes were collected during the enrollment period of the "efficacy study". There were 1011 fleas recovered in environmental flea traps, 281 fleas from cats, and 99 fleas from dogs (Table 1). A total of 113 pools of fleas from 40 traps in 24 homes, 74 pools from 40 cats in 31 homes, and 26 pools from 8 dogs in 7 homes were tested. Of the pools tested, 8/213 pools (3.8%) tested positive by PCR for *D. caninum;* 6/113 (5.3%) of pools from traps, 2/74 (2.7%) of pools from cats, and 0/26 (0%) of pools from dogs. There were no homes with more than one positive pool from the traps or cats, and only one home (Home 39) had a pool from a trap and cat both tests positive. There is no significant difference between the percentage of positive pools from traps, cats, or dogs ($X^2 < 1.45$, $p > 0.22$). The Minimum Infection Rate (MIR) calculated for the total 213 pools of fleas is 1.25% (Std. Error = 0.44). In homes where pools of fleas were tested, there was a significant difference in the total number of pools tested (trap + cat) in homes with at least one positive pool (Mean = 11.17) and homes with no positive flea pools (Mean = 7.17) (U = 18.5, $p = 0.017$). There was not a significant difference between the mean trap flea numbers of positive (Mean = 36.6) and negative homes (Mean = 43.9) (U = 39.0, $p = 0.317$).

Additionally, 65 individual fleas from environmental flea traps in 37 homes were also tested with only one flea tested from 20 homes, two fleas from 9 homes, three fleas from 5 homes, and four fleas from 3 homes (Table 1). Of the individual fleas tested, 4/65 (6.2%) tested positive for *D. caninum*, including two fleas from the same trap in Home 24. There was no significant difference between the percentage of pools testing positive for *D. caninum* and individual fleas tested ($X^2 = 0.69$, $p = 0.405$). All of the positive individual fleas were collected from homes with a positive pool of trap fleas (Homes 13, 24, and 41).

All *Dipylidium caninum* sequences from positive pools and individual fleas most closely matched published feline genotypes (MH045477: 100.00% identity) and were more dissimilar to canine genotypes (MH04566: 96.17% identity).

Table 2.1 Description of the total fleas, traps, cats, pools of fleas, and individual fleas collected from each home.

Home	Number of fleas collected in traps	Number of traps with fleas	Trap pools tested	Number of cats with fleas	Cat pools tested	Number positive pools	Total pools tested	Individual fleas tested
1	25	2	5	2	4	-	9	3
2	-	-	-	-	-	-	-	1
3	-	-	-	1	2	-	2	-
4	-	-	-	1	2	-	2	-
5	-	-	-	-	-	-	-	1
6	-	-	-	1	**1***	1	1	-
7	-	-	-	1	3	-	3	2
8	59	2	7	1	3	-	10	3
9	-	-	-	-	-	-	-	1
10	-	-	-	1	1	-	1	-
11	6	1	2	1	2	-	8	1
12	18	1	**4***	2	3	1	7	2
13	20	1	**4***	1	1	1	9	**4***
14	-	-	-	-	-	-	-	1
15	10	2	2	1	1	-	3	2
16	17	2	5	1	1	-	6	1
17	-	-	-	1	1	-	1	-

18	-	-	-	-	-	-	-	1
19	38	2	8	1	3	-	11	4
20	-	-	-	1	1	-	1	-
21	4	1	1	-	-	-	1	1
22	27	1	4	3	9	-	13	3
23	-	-	-	1	1	-	1	-
24	34	2	**7***	3	5	1	16	**3****
25	-	-	-	-	-	-	-	1
26	99	2	7	1	1	-	8	2
27	-	-	-	-	-	-	-	1
28	-	-	-	-	-	-	-	2
29	13	2	4	2	4	-	8	1
30	-	-	-	-	-	-	-	1
31	17	2	5	-	-	-	9	2
32	10	2	3	2	2	-	8	1
33	33	2	5	-	-	-	5	3
34	-	-	-	1	2	-	2	1
35	387	2	8	1	1	-	9	4
36	-	-	-	1	1	-	1	-
37	-	-	-	1	1	-	1	-
38	-	-	-	1	1	-	1	-
39	42	2	**8***	2	**6***	2	14	2
40	7	1	**2***	1	4	1	10	1
41	99	2	**8***	1	3	1	11	**2***
42	-	-	-	-	-	-	-	1
43	7	1	2	1	1	-	6	2
44	12	1	4	-	-	-	4	1
45	20	2	6	-	-	-	6	1
46	-	-	-	-	-	-	-	1
47	7	2	2	1	3	-	5	1
Totals	1011	40	113	40	74	8	213	65

*Indicates a positive PCR test for *Dipylidium caninum*
**Indicates two positive PCR tests for *Dipylidium caninum*

2.5 Discussion

Dipylidium caninum remains an important parasite affecting dogs and cats worldwide. Recent reports of praziquantel resistance highlight the need for multimodal prevention focusing on effective flea control, and yet, the lack of prevalence data for fleas and *Dipylidium caninum* in fleas leaves pets at unknown risk levels. In this study, we were able to document the percent of *D. caninum*-infected fleas in a small geographic region in west central Florida, which is the first description of infection prevalence in North America. This was a cat-focused product efficacy study where fleas were opportunistically collected from infested pets and home environments. Despite having significant flea infestations, no owners reported seeing proglottid shedding at the time of enrollment, although pets in Home 44 were later diagnosed with *D. caninum* infections. This is of note as all of the 4 flea pools and 1 individual flea tested negative for *D. caninum*.

The percent of positive trap pools (5.3%), cat pools (2.7%), and individual fleas (6.2%) are similar to the few published of *D. caninum* in cat fleas. The previous studies described 2.23% of individual *C. felis* from cats and 5.2% from dogs testing positive for *D. caninum* (Beugnet et al., 2014). Several studies looked at pooled prevalence, although the pool sizes were variable. Low et al. tested 42 individual fleas and 10 pools of 3 – 8 fleas (Total fleas = 92) and determined a MIR = 2.2% (Low et al., 2017). Similarly, Abdullah et al. tested pooled fleas for *D. caninum* but by flea species and animal. While there is no exact description of the pool sizes, 13/429 pools tested positive for *D. caninum,* although no MIR was calculated from the pooled testing (Abdullah et al., 2019). Therefore, the MIR calculated from this study (1.25%) represents the lower bound of the reported prevalence of *D. caninum* in the cat flea. The pool size of 3 fleas was chosen for a separate study focusing on *Bartonella* spp. and *Rickettsia* spp., but this pool size may be appropriate for larger surveillance efforts given there was no significant difference between the percent of positive pools from the environment (5.3%) and individual fleas (6.2%).

There were no significant differences between the percent of positive flea pools from traps and cats ($p = 0.22$) suggesting that the prevalence of *D. caninum* remains at a low level in fleas throughout the infested environment. Given that significantly more fleas were collected from the home environment than from animals, testing more trap-collected fleas could increase the sensitivity of testing compared to lower numbers of on-animal fleas. This is highlighted by the fact that the likelihood a household would have a *D. caninum* positive at all was correlated with the number of pools tested and not total fleas collected. Increasing the total pools tested is more feasibly done through testing increased numbers of trap pools than enrolling more cats in a study.

The main efficacy portion of this study was focused on the efficacy of lotilaner in controlling fleas on cats (Sutherland et al., In Review), and therefore the household demographics were skewed to include more cats than dogs. While some fleas were collected from dogs, there were significantly more pools of fleas tested from cats. Because of the low number of flea pools tested, there was no difference between the percent of positive fleas from cats (2.7%) and dogs (0%) ($p = 0.4$). Additionally, all of the positive *D. caninum* samples most closely matched reported published sequences for the feline genotype found in the United States, Europe, and South Africa (Labuschagne et al., 2018). Further research focusing on fleas collected from dogs would be needed to understand any prevalence or genotype differences from fleas collected from cats and dogs in Florida.

2.6 Conclusions

Dipylidium caninum is a worldwide parasite, seemingly found in approximately 2 – 5% of cat fleas. This represents an ever-present risk to dogs, cats, and humans. Testing pools of fleas may be an efficient method for surveillance of *D. caninum*, not just for prevalence, but potentially genotype variation and drug resistance markers in the future. Overall, more studies are needed to determine if the percentage of cat fleas infected with *D. caninum* varies geographically, seasonally, or by vertebrate host. Given the unknown risk of *D. caninum* from fleas and the variable seasonality of fleas across North America, pets should be kept on high-quality flea control as an additional measure to not only prevent flea infestations, but also infection with the flea tapeworm.

Chapter 3 - First detection of *Echinococcus multilocularis* in wild canids in Kansas and Missouri, a new endemic region & an update Illinois and Indiana, previously endemic regions

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3.1 Abstract

Background: *Echinococcus multilocularis* and *Echinococcus granulosus* sensu lato are zoonotic tapeworms of wild canids that also infect humans and domestic animals. The number of sporadic human and animal cases of *Echinococcus* spp. infections being reported in North America is driving a need to address the gap in wildlife surveillance of these important cestodes.

Methods: Coyotes are routinely trapped for fur trading and nuisance control. Carcasses were collected from Kansas (n = 53), Missouri (n = 52), Illinois (n = 50), and Indiana (n = 49) and processed. Intestinal tracts were removed, frozen at -80°C for at least 7 days, and then processed by sifting, filtration, and counting technique (SFCT) to identify adult *Echinococcus* spp. Positive samples were morphologically and molecularly identified using PCR targets for *nad1* and *rrnS* genes.

Results: *Echinococcus* spp. adults were recovered from 25/53 coyote from KS, 22/52 from MO, 10/50 from IL, and 8/49 from IN, with an overall prevalence of 31.4%. All, but one, of positive samples were morphologically and molecularly identified as *E. multilocularis*, with sequences closely matching isolate E4 using the *nad1* and *cob* gene. The was one positive sample molecularly identified as *E. granulosus* from a coyote in Indiana, U.S.A.. Sufficient feces were obtained from 197 coyotes, 144 of which had adult *Taenia* spp. and/or *Echinococcus* spp. found during SFCT. Taenia-type eggs were recovered from 51/143 adult-positive samples giving a sensitivity of 35%. Of the 51 fecal samples positive for taenia-type eggs, 14 had *Echinococcus* spp. adults only, 21 had Taenia spp. adults only, and 16 were coinfections. Additionally, taenia-type eggs were only found in 1/54 adult-negative samples.

Conclusion: As the first description of *E. multilocularis* in wild canids in Kansas, and the first systematic description of *E. multilocularis* in Missouri, this study confirms the expanding range

of *E. multilocularis*. Because coyotes and other wild canids can serve as a source of infection for domestic dogs and humans, these findings demonstrate a growing zoonotic threat, given the increase in urban and peri-urban coyote populations. Investigation of additional definitive and intermediate hosts in historically non-endemic areas is warranted to gain further insights into this growing zoonotic threat.

3.2 Background

Echinococcus multilocularis and *Echinococcus granulosus* sensu lato are zoonotic tapeworms with definitive hosts that include wild canids, such as red foxes (*Vulpes vulpes*), coyotes (*Canis latrans*) and domestic dogs. The eggs of *Echinococcus* spp. are resistant in the environment, and under optimal conditions can remain infective for more than a year (Eckert et al., 2001). After ingestion by intermediate hosts the eggs hatch to release oncospheres that penetrate the intestinal wall and migrate to internal organs (Eckert et al., 2001). Humans and dogs become aberrant, intermediate hosts after inadvertent ingestion of infectious eggs. Infectious eggs may be ingested from contaminated soil or vegetation or activities and occupations with significant exposure to canid wildlife or carcasses (Cerda et al., 2018; Lavallée-Bourget et al., 2024; Nunnari, 2012; Polish et al., 2021). This presents a significant public health concern because of the zoonotic alveolar and cystic hydatid disease caused by *E. multilocularis* and *E. granulosus*, respectively (Eckert et al., 2001; Frey et al., 2017).

Reports of human and canine echinococcosis in Canada indicate a geographic expansion of the range for *E. multilocularis* (Kotwa et al., 2019; Lavallée-Bourget et al., 2024; Peregrine et al., 2012). The increase in cases appears to coincide with reports that the emerging strains most closely match pathogenic European haplotypes (Massolo et al., 2019; Polish et al., 2021). Historically, in the Midwest United States the North American N2 haplotype of *E. multilocularis* was endemic with 18 - 21% of wild canids infected in Illinois and Indiana in the 1980s and 1990s (Ballard & Vande Vusse, 1983; Storandt & Kazacos, 1993). Unpublished data in red foxes provided evidence of endemicity in Missouri, while, in Kansas 111 wild canids were tested and none were positive . In the United States, the first report of canine alveolar echinococcosis outside the known historic endemic range for *E. multilocularis* was in a domestic dog in Virginia (A. Zajac et al., 2020). More cases of alveolar echinococcosis in domestic dogs were reported from Missouri, Washington, and Kansas, as well as cases of intestinal echinococcosis of a domestic canine was reported in Missouri, Colorado, Kansas, Idaho, Illinois, Montana, Nevada, Oregon, Washington, and Wyoming (Evans et al., 2006; Kuroki et al., 2020, 2022; Williams & Walzthoni, 2023).

Surveillance of domestic animals for infection with *Echinococcus* spp. is complicated and unrewarding, as antemortem diagnosis is difficult. A positive fecal flotation may be diagnosed as taeniid egg positive without further pursuit of specific identification, and until recently, routine PCR testing of canine fecal samples for parasites like *Echinococcus* spp. was not common (K. Gesy, Pawlik, et al., 2013; E. J. Jenkins et al., 2023; Villeneuve et al., 2015b). With increases in urban and peri-urban interactions of coyotes with pet dogs and humans, there is a need for surveillance of the associated growth in risk of echinococcosis (C. A. Thompson et al., 2021). To provide insight into this risk, this study was initiated to investigate the occurrence of *E. multilocularis* by collecting and examining carcasses of coyotes in the Midwest United States.

3.3 Methods

3.3.1 Sample Collection

All animal use was conducted under Kansas State University IACUC #4944, in which no animals were harvested specifically for this project. Coyote carcasses were opportunistically collected after being discarded by licensed trappers and hunters in Kansas, Missouri, Illinois, and Indiana. Necropsies and carcass disposal were carried out at the Kansas State University Veterinary Diagnostic Laboratory. Standard necropsies were performed on all well-preserved carcasses with an intact abdominal wall and gastrointestinal tract. Intact intestinal tracts were double-ligated using twine, double-bagged in sealable bags, and preserved at -80ºC for a minimum of 7 days to inactivate any taenia-type eggs present in the sample (Eckert et al., 2001).

3.3.2 Museum Catalog for Host Specimens

Given that studies on parasites and pathogens invariably lead to extended research questions, we prepared host voucher specimens in the form of complete skull, or limb long bones (where skull was damaged or absent) and cataloged these, along with cryopreserved muscle tissue in the Kansas State Biorepository to facilitate future studies related to our findings (Galbreath et al., 2019; C. W. Thompson et al., 2021). All archived vouchers and tissues can be accessed through the Arctos digital specimen database (https://arctos.database.museum/) and museum catalog numbers for all host specimens have been provided in Appendix B.

3.3.3 Sifting, filtration, and counting technique (SFCT)

After egg inactivation, the intestines were thawed overnight at 4ºC, arranged from pylorus to anus, and cut into four sections (K. Gesy, Pawlik, et al., 2013). The two distal sections were squeezed to extract any feces present into sample cups, to be used for flotation and PCR analysis. The four sections were then longitudinally cut to expose the internal lining and individually shaken in a glass jar with 250mL of water. To remove any remaining intestinal lining, the intestines were scraped between two fingers into the glass jar and then discarded. The contents of the glass jar were then filtered through a 1.4 mm mesh sieve (Fisher Scientific Company, No. 14; Pittsburgh, United States) to remove any macroscopic helminths or debris.

The filtrate was then sieved through a 150 µm mesh sieve (Dual MFG Co., No. 100; Chicago, Illinois, United States) to collect *Echinococcus* spp. adults or segments. The contents in the second sieve were back rinsed into a 50mL falcon tube using water. All intestinal contents were microscopically evaluated for *Echinococcus* spp. adults in 3mL aliquots. If *Echinococcus* spp. adults were found, the sample was deemed positive, adults were morphologically identified (Heidari et al., 2019), and a subset of adults was preserved in 70% EtOH for molecular testing.

3.3.4 PCR: *Echinococcus* spp. adults

For all samples positive for *Echinococcus* spp. adults, three individual adults and three pools of five intact adults were collected and stored in 70% EtOH at -20ºC. Individual adults and pools were processed using Zymo Research Quick-DNA Miniprep Kit (Zymo Research; Irvine, California, United States) following the Cell Suspensions and Proteinase K Digested Samples protocol with modifications. Briefly, first the *Echinococcus* spp. adults and pools were digested in a solution of 95 µL Genomic Lysis Buffer (Zymo Research; Irvine, California, United States), nuclease-free water, and Proteinase K, 5 µL for individuals and 10 µL for pools. Then, following kit instructions, the adults in solution were incubated in a dry bath at 55ºC for 2 hours prior to DNA extraction. Adults were molecularly identified using a multiplex PCR targeting *nad1* for *Echinococcus multilocularis*, *rrnS* for both *Echinoccocus granulosus*, and *Taenia* spp. (see Table 3.1) (Trachsel et al., 2007). PCR reactions were performed in a 50 µL solution of 25 µL GoTaq Green, 18 µL Nuclease-free water, 1 µL of each primer (Cest 1-5), and 2 µL of sample gDNA.

Table 3.1 PCR reaction targeting the *nad1* and *rrnS* gene of *E. multilocularis* and *Taenia* spp., respectively to determine the species of taeniid DNA **(Trachsel et al., 2007)**.

Target species	**Gene**	**Primer name**	**Sequence (5'-3')**	**Amplicon size**	**Cycling conditions**
E. multilocularis	*nad1*	Cest 1	TGCTGATTTGTTAAAGTTA GTGATC	395 bp	94°C for 30sec
		Cest 2	CATAAATCAATGGAAACA ACAACAAG		

E. granulosus	*rrnS*	Cest 4	GTTTTTGTGTGTTACATTA ATAAGGGTG	117 bp	58°C for 90sec 72°C for 10sec Repeat for 40 cycles
		Cest 4	GCGGTGTGTACMTGAGCT AAAC		
Taenia spp.	*rrnS*	Cest 3	YGAYTCTTTTTAGGGGAA GGTGTG	267 bp	
		Cest 5	GCGGTGTGTACMTGAGCT AAAC		

3.3.5 PCR: *Echinococcus* spp. Haplotyping

To further genetically characterize cestodes for phylogenetic analyses, we evaluated additional mitochondrial DNA (mtDNA) genes to determine the haplotype. PCR was performed as above using primers designed by Gesy and Jenkins (2015) for the cytochrome b (*cob*) and NADH dehydrogenase subunit 2 (*nad2*) gene (K. M. Gesy & Jenkins, 2015). Nucleotide sequences were concatenated manually using BioEdit (Hall, 1999, 2011). Multiple alignment files in PHILIPS and NEXUS formats were prepared in AliView (Larsson, 2014). The resulting sequences were compared and aligned to published haplotypes from Asia, Europe, and North America. A maximum likelihood phylogenetic tree with 1000 bootstraps was conducted using IQ Tree (Hoang et al., 2018; Nguyen et al., 2015). The best-fit substitution model for the data set was Hasegawa-Kishini-Yano model using a discrete gamma distribution and assuming a certain fraction of sites is evolutionary invariable (KHY + G + I). *Taenia saginata* was used as an outgroup.

Table 3.2 PCR reaction targeting the *nad2* and *cob* gene of *E. multilocularis* to determine the haplotype (K. M. Gesy & Jenkins, 2015).

Gene	Primers set (5' – 3')	Amplicon parameters	Amplicon	
			bp	nt
NADH dehydrogenase subunit 2 (*nad2*)	F: GGGTTTTTTTGGAGTTGTG R: AAGGCATAGAYACAGGAGTCA	95 C for 3 min; (94 C for 30 s, 54 C for 30 s,	623	104 - 727

Cytochrome B (*cob*)	F: TGCGTTATTGGCATATGGTAG R: GTGCCACCCTCAGTTGGTACT	72 C for 45 s) x 40; 72 C for 5 min	693	209 - 902

3.3.6 Fecal testing: Flotation and PCR

Standard double centrifugal fecal flotations using Sheather's sugar (S.G. 1.27) were performed to recover eggs from ~2 – 5 grams of feces (A. M. Zajac et al., 2021). Any taeniid eggs that were recovered were washed off the slide into a 50mL falcon tube using distilled water. The tube was centrifuged at 1000 rpm, the supernatant was decanted, and the pellet preserved in 70% EtOH at -20ºC for PCR. If taeniid eggs were not present, then the slide was discarded. Taeniid eggs recovered during flotation were identified to species through molecular testing. A QIAamp® Fast DNA Stool Mini Kit (Qiagen, Cat. No. 51604; Hilden, Germany) was used for DNA extraction following the "For Pathogen Testing" protocol with a modification of adding 200uL of InhibitEX buffer to the samples prior to lysis in a dry bath at 70ºC for 5 minutes. For PCR identification of taenia-type eggs, a single PCR reaction targeting the NADH dehydrogenase subunit 1 (*nad1*) mitochondrial gene of *E. multilocularis* and the small subunit of ribosomal RNA (*rrnS*) of *Taenia* spp., respectively were used (see Table 3.1) (Trachsel et al., 2007).

All PCR reactions were visualized on a 2% agarose gel and bands were visualized using EZ-Vision® Three. DNA was purified from positive PCR reactions using Promega Wizard SV Gel and PCR Clean-Up (Promega; Madison, WI), and sent to Eurofins (Eurofins; Des Moines, USA) for sequencing. Sequences were compared to published targets on GenBank (Appendix C).

3.4 Results

From December 2021 to February 2024, 206 wild canid carcasses were collected. In total, there were 53 coyotes from 22 counties in Kansas, 52 from 22 counties in Missouri, 50 from 10 counties in Illinois, and 49 from 4 counties in Indiana. There were 2 additional red fox carcasses, one from Kansas and one from Missouri.

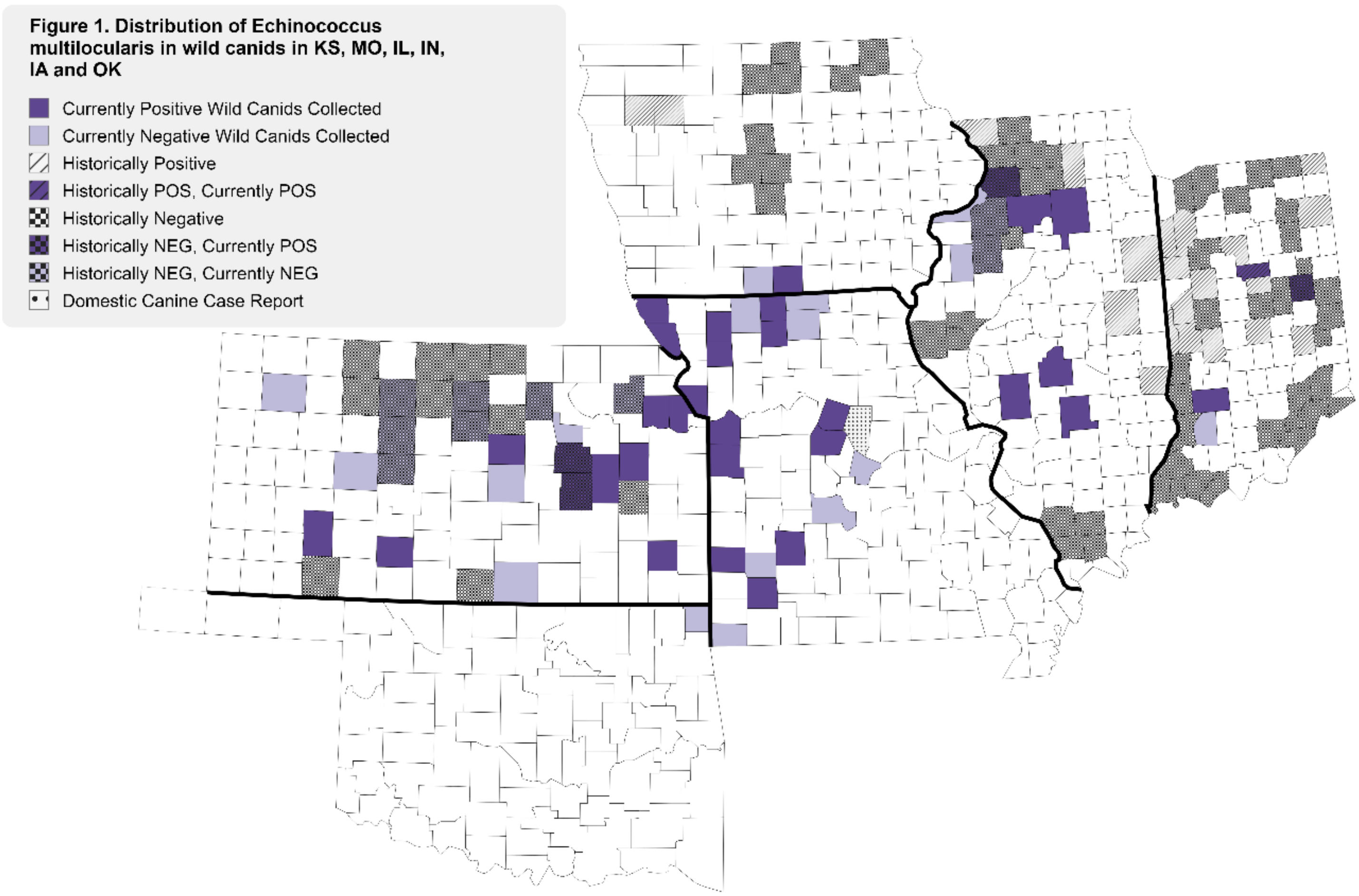

Figure 9 Distribution of *Echinococcus multilocularis* in wild canids in Kansas, Missouri, Illinois, Indiana, Iowa, and Oklahoma. Map includes historical wild canid surveillance and current surveillance from this study (Ballard & Vande Vusse, 1983; Dyer & Klimstra, 1980, 1981; Leiby et al., 1970; Storandt et al., 2002; Storandt & Kazacos, 1993).

From SFCT, *Echinococcus* spp. adults were collected in 65/204 (31.8%) of coyotes and adult *Taenia* spp. from 122/204 coyotes. Adult *Echinococcus* spp. were found in 25/53 (47.1%) coyotes from Kansas, 22/52 (42.3%) from Missouri, 10/50 (20%) from Illinois, and 8/49 (16%) from Indiana; and one red fox 1/1 (100%) from Jackson County in Missouri (Figure 8). Only

Echinococcus spp. were recovered from 29/204 coyotes, while 36/204 were co-infected with both taeniid adults, and 86/204 were infected with *Taenia* spp. adults (see Table 3.2). For report of additional internal and external parasites found, see Appendix A. Significantly more *Echinococcus* spp. adults were recovered from coyotes from KS and MO coyotes compared to IL and IN ($X^2_{\mathrm{KS/IL}(1,\, N = 103)} = 8.46$, $p = 0.003$; $X^2_{\mathrm{KS/IN}(1,\, N = 102)} = 11.07$, $p = 0.0009$; $X^2_{\mathrm{MO/IL}(1,\, N = 102)} = 5.89$, $p = 0.015$; $X^2_{\mathrm{MO/IN}(1,\, N = 101)} = 8.15$, $p = 0.004$). States with a lower percent of *Echinococcus* spp. positive samples (Illinois and Indiana) had a statistically significant higher percent of *Taenia* spp. positive samples than states with a higher percent of *Echinococcus* spp. positive samples (Kansas and Missouri) ($X^2_{(1,\, N = 204)} = 15.54$, $p < 0.0001$). This difference in *Taenia* spp. positive samples is exacerbated when co-infections are removed and individual tapeworm infections are compared between low *Echinococcus* spp. states ($\mathrm{IN/IL}_{\mathrm{Echino}} = 4\%$; $\mathrm{IN/IL}_{\mathrm{Taenia}} = 53\%$) and high *Echinococcus* spp. states ($\mathrm{KS/MO}_{(\mathrm{Echino})} = 23\%$; $\mathrm{KS/MO}_{(\mathrm{Taenia})} = 25\%$) ($X^2_{(1,\, N = 204)} = 23.99$, $p < 0.0001$).

Table 3.3 Number of *Echinococcus* spp., Co-infection, and *Taenia* spp. only coyote samples collected from Kansas, Missouri, Illinois, and Indiana

Tapeworm Status	**Number of coyote samples by state**				
	Kansas	**Missouri**	**Illinois**	**Indiana**	**Total**
Echinococcus spp. only	13	12	2	2	29
Co-infection	12	10	8	6	36
Taenia spp. only	11	16	31	28	86
No adult tapeworms recovered	17	14	9	13	53
Total coyotes collected	53	52	50	49	204

There were enough adult worms for full PCR testing (3 individual worms and 3 pools of 5 worms) in 44/66 coyotes. Using PCR, all *Echinococcus* spp. adults in KS, MO, and IL were molecularly confirmed as *E. multilocularis* (% nucleotide match; GenBank accession nos.), and only one coyote in IN was infected with *E. granulosus.* The haplotype of *E. multilocularis* most closely matches a European haplotype reported in Poland as well as recently in Alberta, Canada (Figure 9).

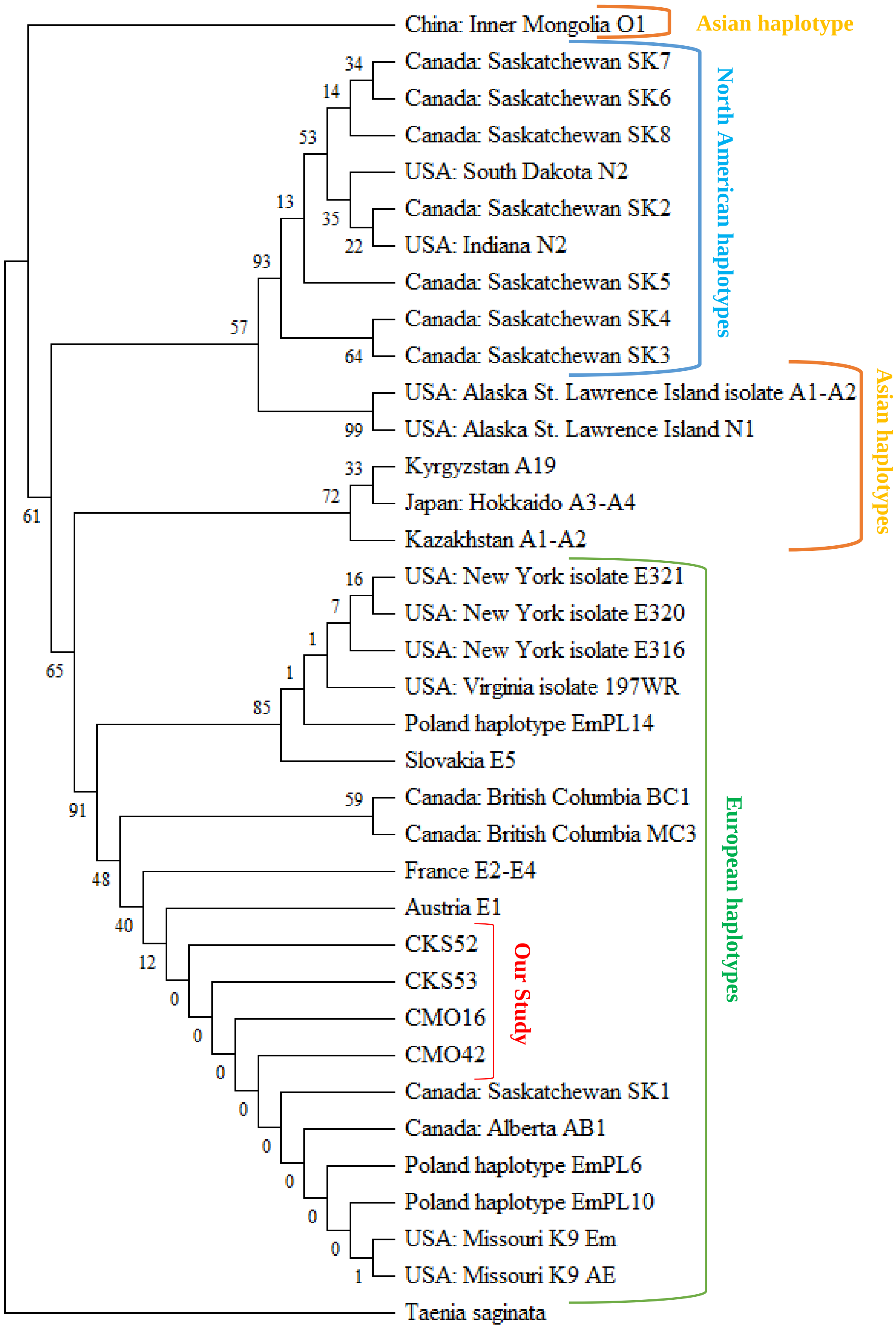

Figure 10 Phylogenetic tree of *Echinococcus multilocularis* haplotypes based on concatenated sequences of the *nad2* and *cob* genes. Values on the tree nodes represent bootstrap values. Representative sequences from our study are identified by a red bracket. *Taenia saginata* was used as an outgroup.

From the SFCT procedure, sufficient feces were recovered from 197 coyotes (50 from KS, 51 from MO, 49 from IL, 47 from IN) for flotation examinations to detect taeniid ova (see Table 3.3). Because both *Taenia* spp. and *Echinococcus* spp. tapeworms produce morphologically similar taeniid eggs, comparison of fecal flotation results analyzed samples with either adult tapeworm. Taenia-type eggs were found in fecal samples from 51/143 (35%) coyotes infected with taeniid adults and there was no significant difference in recovery of eggs from adult positive samples between any of the states ($X^2 < 0.71$; $p > 0.40$). Of the 50 samples that were adult and egg positive, significantly more of those samples had *Echinococcus* spp. adults recovered (only or co-infection) compared to *Taenia* spp. only or co-infection ($X^2_{(1, N = 177)} = 8.61$ $p = 0.003$). Again, removing samples that were co-infected, there were significantly more taeniid egg positive samples from coyotes infected with only *Echinococcus* spp. compared to only *Taenia* spp. ($X^2_{(1, N = 109)} = 8.61$; $p = 0.002$). One sample from Indiana was positive for taenia-type eggs despite not finding any intestinal cestode adults. Those eggs were molecularly identified as *Echinococcus multilocularis* by PCR.

Table 3.4 Total number of fecal samples tested for taenia-type eggs from *Echinococcus* spp., Co-infection, *Taenia* spp., and coyote samples with no taeniid adults.

	***Echinococcus* Adults Only**	**Co-infection**	***Taenia* Adults Only**	**No Adults**	**Total**
Fecal Float positive	14	20	17	1	52
Fecal Float negative	14	14	64	52	144
Total	28	34	81	53	197

3.5 Discussion

This study reports the first positive cases of *Echinococcus multilocularis* in coyotes in both Kansas and Missouri. Previous research in Kansas found 0/89 coyotes and 0/22 red foxes infected with *Echinococcus* spp., and in that same study 27/72 red foxes in Nebraska tested positive for *Echinococcus multilocularis* but 0/31 coyotes were infected (Storandt et al., 2002). The positive red foxes were all from northern Nebraska above the 41st parallel line. It was speculated at that time that since coyotes have a much broader diet than red foxes, the data suggested that the prevalence in rodents was likely very low or absent in many areas of Kansas and Nebraska. No previous surveillance of wild canids in Missouri had been conducted although an intestinal and alveolar case of echinococcus in a dog were reported in 2020 and 2022 respectively (Kuroki et al., 2020, 2022). Given that >40% of coyotes from Kansas and Missouri both had *E. multilocularis* adults, this may represent both a southern expansion of the range as well as a shift in the predominant definitive host, although there were not enough red foxes included in this study to evaluate that claim. Storandt & Kazacos (1993), also reported red foxes and coyotes infected with *E. multilocularis* in Indiana and Illinois, but again, all positive cases were in the northern and central portions of the state about the 39th parallel. The one coyote that was positive for *E. granulosus* was from Davies County in southern Illinois, outside of the traditional range. Taken together, the findings of this study further raise the concern about the increasing prevalence of this parasite and the associated risk of alveolar echinococcosis in humans and domestic animals. Furthermore, as coyotes are reported to have established populations in most urban areas across North America, the zoonotic risk clearly extends to non-rural areas (Catalano et al., 2012; C. A. Thompson et al., 2021).

To our knowledge, this is the first study to compare *Echinococcus* spp. and *Taenia* spp. infections in coyotes. The inverse relationship between the two tapeworms seen in this study might suggest differences in diets between coyotes tested in Kansas and Missouri compared to Illinois and Indiana, although diet analysis and species identification of the *Taenia* spp. tapeworms were outside the scope of this study. Further analysis of the samples might provide more insight on these relationships.

Microscopic detection of cestode infections by fecal flotation is recognized as having low sensitivity, and yet, is still considered the primary diagnostic technique used in domestic dogs (Conraths & Deplazes, 2015; A. Zajac et al., 2020). The low sensitivity (35%) of the fecal flotation to detect taeniid adult infections is similar to previous research from Canada that found taeniid eggs were recovered by fecal flotation in 23 – 25% of coyotes infected with adult tapeworms (Kolapo et al., 2021a). The poor recovery of taeniid eggs can be due to multiple factors including the presence of prepatent infections, few eggs being produced when infection intensity is low, eggs not being released from proglottids, and taeniid eggs being too heavy to recover with traditional flotation solutions. Both fecal PCR and fecal sedimentation have been shown to be more sensitive methods of detecting taeniid adult infections, both of which will be discussed in Chapter 4 (Kolapo et al., 2021a; Yasur-Landau et al., 2023).

3.6 Conclusion

This is the first description of *E. multilocularis* in wild canids in Kansas and Missouri, as well as an updated surveillance on the parasite in Illinois and Indiana. This study documents the expanding known range of *E. multilocularis* in the US, warranting further investigation of additional definitive and intermediate hosts in historically non-endemic areas. Because coyotes and other wild canids can serve as a source of infection for domestic dogs and humans, these findings demonstrate a growing zoonotic threat given the increase in urban coyote populations.

Chapter 4 - The Hardship of Diagnostics: Comparison of Diagnostic Techniques for the Detection of *Echinococcus multilocularis* in Wild Canid Feces

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4.1 Abstract

Background: Taeniid tapeworms, like *Taenia pisiformis* and *Echinococcus multilocularis*, infect domestic dogs worldwide. All taeniid tapeworms produce a morphologically similar egg that cannot be differentiated between zoonotic *Echinococcus* spp. and other commonly seen *Taenia* spp., such as *T. pisiformis* and *T. taeniaeformis* that are not zoonotic. Since *Echinococcus* spp. are a major public health threat to domestic and dogs, reliable diagnostics tests are needed for surveillance and diagnosis of these zoonotic parasites.

Methods: Coyotes are routinely trapped for fur trading and nuisance control. Carcasses were collected, processed, and intestinal tracts were analyzed using the sifting, filtration, and counting technique (SFCT) to identify taeniid adults. Fecal material was collected during the intestinal analysis from the distal half of the intestines and preserved in 70% ethanol at 4°C. A double centrifugal fecal flotation and sedimentation was performed to identify taenia-type eggs. Copro-PCR, was also performed, targeting the *nad1* and *rrnS* genes.

Results: Taeniid adults were collected from 151/204 coyotes in the Midwest United States, with an overall prevalence of 31.4%. Sufficient feces were obtained from 157/204 coyotes such that two fecal flotations and sedimentation could be performed. Of those 157 samples, 113 had adult *Taenia* spp. and/or *Echinococcus* spp. found during SFCT. Taenia-type eggs were recovered from 43/113 (38%) (CI 26 – 51) adult positive samples by fecal flotation and 46/113 (40%) (CI 31 – 50) adult positive samples by sedimentation. The combined overall sensitivity for centrifugal fecal flotation and sedimentation was 54/113 (47%). Sufficient feces were obtained from 89 coyotes to additionally run a copro-PCR, with 62/89 of those samples positive for *Taenia* spp. and/or *Echinococcus* spp. adults. Taenia-type eggs were recovered from 20/62 (32%) (CI 26 – 51) adult-positive samples on centrifugal fecal flotation, 26/62 (41%) (CI 29 –

55) samples were positive on sedimentation, and 20/62 samples (32%) (CI 21 – 45) were positive on copro-PCR. The combined sensitivity for all fecal techniques compared to adult recovery was 32/62 (51%) (CI 38 – 64).

Conclusions: Adult cestode recovery remains the gold standard for the diagnosis of *Echinococcus multilocularis* in coyotes. A float/sediment may improve taenia-type egg recovery. There is still a need to find a reliable method for extracting DNA from taenia-type eggs.

4.2 Introduction

Taeniid tapeworms, *Taenia* and *Echinococcus* spp., have predator-prey life cycles, and live in the small intestines of wild and domestic canids and felids. The two *Echinococcus* spp., found globally are *Echinococcus granulosus* and *E. multilocularis*, zoonotic tapeworms that infect wild and domestic canids and felids, as well as humans in North America. Cystic and alveolar echinococcosis, caused by *E. granulosus* and *E. multilocularis*, respectively, are neglected zoonotic diseases (NZDs) in humans that are potentially fatal if left untreated (WHO, 2021). *Taenia* species found in North America include *T. crassiceps, T. hydatigena, T. krabbei, T. polycantha,* and *T. twitchelli* (Bouchard et al., 2021). Despite not being zoonotic to humans, these species have veterinary importance because taeniid tapeworms produce a morphologically similar egg, a taenia-type egg, that cannot be identified by the genus level (Lightowlers et al., 2021; Varcasia et al., 2022). Additionally, taenia-type eggs are immediately infectious once shed in the environment and can remain viable for up to one year (Romig et al., 2017).

Domestic dogs can serve as definitive hosts for *E. multilocularis.* Endemic regions can have an established domestic life cycle pattern involving domestic dogs and rodents, where domestic dogs serve as the main source of contamination of infectious taenia-type eggs in the environment. Therefore, domestic dogs can be used to assess the prevalence of *E. multilocularis* in these endemic regions (Romig et al., 2017). Domestic dogs can also be used for overall disease control and prevention because they are prime targets for prevention strategies such as deworming (P. Craig et al., 2015; P. S. Craig et al., 2017).

Gastrointestinal parasitic infections are commonly diagnosed by fecal exams including fecal smears, sedimentations, and centrifugal fecal flotations. Studies have shown that tapeworm infections often go underdiagnosed in domestic dogs (Little et al., 2015). Proglottids can be difficult to find on fecal examination because they are fragile and susceptible to desiccation (Elsemore et al., 2023). Therefore, tapeworm infections are typically diagnosed by visualizing proglottids in the feces, perianally, in the fur, or in the environment.

The gold standard for diagnosing *Echinococcus* spp. infections is through post-mortem identification of adults in the intestinal contents of definitive hosts using the sedimentation, counting, and filtration technique (SFCT) (Gesy, et al., 2013). Post-mortem diagnosis has been the most widespread surveillance method in wild canids due to the fact that coyotes and red fixes are often trapped and hunting for fur and nuisance. Ante-mortem diagnosis with arecoline bromide and copro-ELISA are possible but met with challenges. For example, the use of arecoline bromide for purgation can take multiple dosages, cause bowel perforation, and the contents have to be boiled in harmful chemicals before it can be analyzed. The use of coproantigen has also been promising, but the test has low specificity and cross reacts with other taeniid tapeworms; therefore should not be used if samples cannot be confirmed with PCR (P. S. Craig et al., 2017). Because *E. multilocularis* has major health implications for humans and domestic animals, it is important to determine if an animal has an active infection.

The aim of this study was to analyze the detection of taenia-type eggs in feces using centrifugal fecal flotation, sedimentation, and copro-PCR against adult cestode recovery, the gold standard.

4.3 Methods

4.3.1 Coyote Sampling and Intestinal analysis of adult cestodes

All animal use was conducted under Kansas State University IACUC #4944, in which no animals were harvested specifically for this project. Coyote carcass collection and intestinal analysis for adult cestodes using the sifting, counting, and filtration technique (SFCT) were performed as stated in Chapter 3 (section 3.3.1 and 3.3.3).

4.3.2 Fecal Sample Collection

For fecal testing, rectal feces, if present, or intestinal contents were collected as distally as possible. Samples for fecal testing were preserved in aliquots at -20ºC prior to processing and thawed at room temperature. The remaining fecal material was preserved in 70% Ethanol and stored at 4ºC.

4.3.3 Centrifugal Fecal Float & Sedimentation

A standard double centrifugal fecal flotation using Sheather's Sugar (specific gravity 1.27) were performed to recover eggs from ~2-5 grams of feces as described in Zajac et al. (2021). Feces were weighed, 2-5 grams, and homogenized with distilled H_2O (dH_2O) using a wooden tongue depressor. The fecal solution was strained through a tea strainer into a 50mL glass tube and centrifuged at 1000 rpm for five minutes. The supernatant was decanted, and the fecal pellet was resuspended in Sheather's Sugar (SPG 1.27) and homogenized by vortexing. The fecal solution was transferred to a 15mL glass tube and Sheather's sugar was used to fill the tube and form a positive meniscus. An 18 x 18 mm coverslip was placed on the tube and centrifuged at 1300 rpm for five mins then allowed to stand for an additional 10 mins. The coverslip was transferred to a microscope slide and analyzed under a microscope on 10X magnification for parasite ova.

Instead of discarding the sedimented contents from the fecal flotation, a fecal sedimentation was then performed. After fecal flotation, Sheather's sugar was first decanted, and the fecal pellet was resuspended in dH_2O and allowed to sit for 15 – 30 minutes. This process of filling, sedimenting, decanting, and refilling, was repeated until the supernatant was clear. Then, 500 μL of the sediment was removed, placed in a 1.5 mL microcentrifuge tube and preserved at -20°C for gDNA extraction and PCR. To analyze the sediment sample for taenia-type eggs, three slides were created from the sediment and microscopically examined. Any extra sediment was saved in a 1.5 mL microcentrifuge tube and stored at -20°C. For all microscopic examination of taenia-type eggs, technicians were blinded to which samples were positive for *Echinococcus* spp. and *Taenia* spp. adults. Taenia-types eggs were enumerated and recorded, with counting stopping at 100 sample.

4.3.4 Fecal DNA extraction

DNA was extracted from 0.2 mg of feces using the QIAamp® DNA Stool Mini Kit (Qiagen, Cat. No. 51604; Hilden, Germany) and performed per kit instructions with no modifications.

4.3.5 Fecal PCR

For PCR testing of fecal samples, a single reaction targeting the NADH dehydrogenase subunit 1 (*nad1*) mitochondrial gene of *E.multilocularis,* the small subunit of ribosomal RNA (*rrnS*) of *Taenia* spp. and *E. granulosus* were used (see Table 3.1 in Chapter 3) (Trachsel et al., 2007). All PCR reactions were visualized on a 2% agarose gel and bands were visualized using

EZ-Vision® Three. DNA was purified from positive PCR reactions using Promega Wizard SV Gel and PCR Clean-Up (Promega; Madison, WI), and sent to Eurofins (Eurofins; Des Moines, USA) for sequencing. Sequences were compared to published targets on GenBank.

4.3.6 Data Analysis

To calculate sensitivity and specificity for centrifugal fecal flotation, sedimentation, and fecal PCR, adult cestode recovery was used as a reference. Data were only analyzed for samples that were positive of both float 1, float 2, and sedimentation. Data were collated and statistical analysis was performed using BioStat for Excel for the comparison of techniques to estimate Kappa value for agreement and statistical significance using McNemar's Chi Square Statistic for comparison between techniques. A Chi Square analysis was used for the comparison of diagnostic techniques to adult cestode recovery. Then, for fecal PCR analysis data was analyzed for samples positive on both flotations, sedimentation and fecal PCR, with overall diagnostic technique analysis coming from this subset of samples. Statistical analysis was performed using Vassar Stats (http://vassarstats.net/clin1.html) to calculate sensitivity, specificity, positive predictive value, and negative predictive value with 95% confidence intervals for fecal flotation, sedimentation, and PCR.

4.4 Results

4.4.1 General Fecal Results

As stated in Chapter 3 (section 3.4), *Echinococcus* and *Taenia* spp. were collected from 151/204 (74%) coyotes. Only *Echinococcus* spp. were recovered from 29/204 coyotes, while 36/204 were co-infected with both taeniid adults, and 86/204 were infected with only *Taenia* spp. adults.

From SFCT, sufficient feces were collected from 157 coyotes (45 from KS, 44 from MO, 26 from IL, and 42 from IN) to perform two centrifugal fecal flotations and a sedimentation (see Table 4.1). Analyses could not be conducted on 40 fecal samples (3 *Echinococcus* spp. only, 7 co-infections, 21 *Taenia* spp. only, and 9 with no taeniid adults) because insufficient feces were available for sedimentation.

Table 4.1 Number of samples analyzed in coyotes harvested in Kansas, Missouri, Indiana, and Illinois.

Location	Positive			Negative	Total Fecal Samples
	Echinococcus spp. Only	Co-infected	*Taenia* spp. Only		
Kansas	12	9	9	15	45
Missouri	12	8	13	11	44
Illinois	1	5	15	5	26
Indiana	2	6	22	12	42
Total	27	28	59	43	157

4.4.2 Microscopy

Sufficient feces were recovered from 157 coyotes for two fecal flotations and a sedimentation and 113 of those samples came from coyotes with adult *Taenia* spp. and/or *Echinococcus* spp. recovered by SFCT (see Table 4.2). Taeniid eggs are produced by both *Echinococcus* spp. and *Taenia* spp., so comparison of microscopy results analyzed samples with either adult tapeworm. In Chapter 3 (section 3.4), the fecal float sensitivity for float 1 was low 51/143 (35%); therefore, those samples were retested using a float/sediment technique to detect taenia-type eggs. The float sensitivity for float 2 was 24/113 (21%), with no statistical difference in taenia-type egg recovery between the two flotations (kappa value $\kappa = 0.66$) ($X^2 = 1.16$, $p > 0.2$).

A comparison between the number of taenia-type eggs recovered from centrifugal fecal flotation (float 2) and sedimentation was performed. Overall, the total number sedimentation positive samples were 50/157 (31%), with a sensitivity of 46/113 (40%) (CI 31 - 50) for samples from coyotes with taeniid adults. The four samples positive for taenia-type eggs were negative for taeniid adults, with the number of taenia-type eggs recovered on sedimentation ranging from 1-24. Furthermore, there was only one sample that was positive on sedimentation and both fecal flotations, despite not finding any taeniid adults. There was a difference between taenia-type egg recovery on sedimentation when compared to float 2 (kappa value κ = 0.60) ($X^2 = 20.1$, $p < 7.0\text{E-}6$). There was a statistical difference in the mean egg recovery in sedimentation, 50 eggs, and fecal float 2, 24 (U = 655, $P < 0.05$ one-tailed).

To compare the flotation technique to sedimentation technique, all taeniid positive samples that were positive at least once on float 1 and/or float 2 were compared to positive sedimentations (see Table 4.2). Overall, the sensitivity of centrifugal fecal flotations was 43/113 (38%) (CI 29 - 47) and sedimentations were 46/113 (40%) (CI 31 - 50), with no difference in performance between the two techniques (kappa value κ = 0.66) ($X^2 = 1.6$, $p > 0.2$).

In Chapter 3, there was a significant difference in the number of taenia-type eggs recovered from *Echinococcus* spp. only samples compared to *Taenia* spp. only samples. Combining Float 1 and Float 2, there are a total of 43/113 (38%) (CI 29 - 47) taeniid egg positive samples, with 12 *Echinococcus* spp. only, 18 co-infections, and 13 *Taenia* spp. only samples. There is a significant difference in taenia-type eggs recovered from samples that have *Echinococcus* spp. adults (30/55) when compared to *Taenia* spp. adults (31/87) ($X^2_{(1, N=142)} = 4.91$, $p < 0.02$). That difference is magnified when co-infections are removed and comparing *Echinococcus* spp. only (12/26) samples to *Taenia* spp. only (11/56) samples ($X^2_{(1, N=82)} = 6.18$,

$p < 0.01$). The same comparison was made for sedimentation to adult cestode recovery. Overall, sedimentation sensitivity was 46/113 (40%) (CI 31 - 50) in taeniid positive samples, with 11 *Echinococcus* spp. only, 19 co-infections, and 16 *Taenia* spp. only. There was no difference seen in taenia-type egg recovery when comparing *Echinococcus* spp. samples to *Taenia* spp. samples by sedimentation ($X^2_{(1, N = 141)} = 3.14$, $p > 0.07$). Again, removing co-infections and analyzing *Echinococcus* spp. only (11/26) and *Taenia* spp. only samples (16/58) do not affect taenia-type egg recovery ($X^2_{(1, N = 84)} = 1.73$, $p > 0.18$).

Table 4.2 Total number of fecal samples tested for taenia-type eggs by flotation and sedimentation of *Echinococcus* spp. only, Co-infection, *Taenia* spp. only, and coyote samples with no taeniid adults. N=157 fecal samples in which both techniques were performed.

		***Echinococcus* only**	**Co-infection**	***Taenia* only**	**No Adults**
Combined Float 1 & 2*	Pos	12	18	13	1
	Neg	14	11	45	43
Sedimentation	POS	11	19	16	4
	NEG	15	9	43	39

*Represents the total number of taeniid positive samples, positive at least once on centrifugal fecal flotation

Table 4.3 Sensitivity, specificity, and predictive values (95 % CI) for centrifugal fecal flotation and sedimentation with reference to adult cestode recovery as the gold standard.

Test Parameters	**Combined F1/F2***	**Sedimentation**
Sensitivity % (CI)	38 (29 - 47)	40 (31 - 50)
Specificity % (CI)	97 (86 - 99)	90 (77 - 97)
Positive Predictive Value % (CI)	97 (86 - 99)	92 (79 - 97)
Negative Predictive Value % (CI)	38 (29 - 47)	37 (28 - 47)

*Represents the total number of taeniid positive samples, positive at least once on centrifugal fecal flotation

4.4.3 Fecal PCR

Sufficient feces were recovered from 89 samples to perform 2 fecal flotations, sedimentation, and fecal PCR (see Table 4.3). Overall, fecal PCR was able to detect taeniid DNA from 22/89 (24%) of fecal samples with a sensitivity of 20/62 (32%) (CI 21 - 45). Within that subset, fecal flotation technique recovered taeniid eggs from 24/89 (26%) samples and the sedimentation technique recovered taenia-type eggs from 26/89 (29%) of samples positive for taeniid adults. All of the fecal float positive samples were from taeniid adult positive coyotes, while 2 of the sedimentation positive samples were from taeniid adult negative coyotes and the 2 samples were also negative on fecal flotation. There was no difference seen between taenia-type egg recovery in fecal flotation to fecal PCR technique (kappa value κ = 0.64) ($X^2 = 0.33$, $p > 0.56$) or sedimentation to fecal PCR (kappa value κ = 0.44) ($X^2 = 1.8$, $p > 0.17$). However, combining F1, F2, and sedimentation together is statistically more likely to detect taeniid eggs when compared to PCR (kappa value κ = 0.52) ($X^2 = 5.5$, $p < 0.018$). Finally, copro-PCR 20/89 (22%) diagnosed statistically less positive coyote samples when compared to adult cestode recovery 62/89 (69%) ($X^2_{(1,\, N = 89)} = 6.24$, $p < 0.012$).

4.4.4 Comparison of all techniques

Finally, when comparing if a sample was positive on at least one diagnostic test (i.e. flotation vs sedimentation vs fecal PCR) to adult cestode recovery, the gold standard of adult cestode recovery was superior, 36/89 (40%) and 62/89 (69%), respectively. Therefore, taeniid adult recovery performed better than all three techniques combined ($X^2_{(1,\, N = 89)} = 10.5$, $p < 0.001$).

Table 4.4 Total number of fecal samples tested for taenia-type eggs by flotation, sedimentation, and fecal PCR of *Echinococcus* spp. only, Co-infection, *Taenia* spp. only, and coyote samples with no taeniid adults. N=89 fecal samples in which all three techniques were performed.

	Fecal Flotation	**Sedimentation**	**Fecal PCR**	**Positive on any test***
Total taeniid positive samples	62	62	62	62
Echinococcus spp. only	12	11	12	14

Co-infection	8	9	4	10
Taenia spp. only	4	6	4	6
Taeniid adult negative	0	2	2	2

*Represents the total number of taeniid positive samples, positive at least once on centrifugal fecal flotation, sedimentation or fecal PCR

Table 4.5 Sensitivity, specificity, and predictive values (95 % CI) for fecal PCR and a positive on any test with reference to adult cestode recovery as the gold standard.

Test Parameters	Fecal flotation	Fecal Sedimentation	Fecal PCR	Positive on any test*
Sensitivity % (CI)	38 (26 – 51)	41 (29 – 55)	32 (21 - 45)	51 (38 - 64)
Specificity % (CI)	1 (84 – 100)	92 (74 – 98)	92 (74 - 98)	85 (65 - 95)
Positive Predictive Value % (CI)	1 (82 – 100)	92 (75 – 98)	90 (69 - 98)	88 (72 - 96)
Negative Predictive Value % (CI)	41 (29 – 54)	40 (28 – 54)	37 (26 - 50)	43 (30 - 57)

*Represents the total number of taeniid positive samples, positive at least once on centrifugal fecal flotation, sedimentation or fecal PCR

4.5 Discussion

Our study showed the hardship that can accompany parasitology diagnostics when analyzing feces for parasite ova and DNA. Our study showed that fecal sedimentation was better at recovering taenia-type eggs than fecal flotation, and recovery of taenia-type eggs improved when both of these techniques are combined. Fecal PCR performed the worst when compared to fecal flotation and sedimentation in detecting samples positive on adult cestode recovery. The ability to detect adult positive samples using fecal PCR only improved when combining positive samples on fecal flotation and sedimentation. In this present study, adult cestode recovery has higher sensitivity than all techniques when compared individually and combined. These findings support previous reports that prove diagnosing cestode infections using adult cestode recovery is the most reliable method (Eckert, 2003). It has been reported that cestode egg recovery, in general, is difficult when using fecal flotation due to intermittent shedding of eggs, prepatent infections, and egg shedding in discreet proglottids (C. Adolph et al., 2017; Dryden et al., 2005;

Little et al., 2015). The low sensitivity in our fecal flotations was not surprising since our results match previous reports from similar wild canid surveillance for *Echinococcus* spp. (Kolapo et al., 2021a; Yasur-Landau et al., 2023). In fact, our study solidifies the difficulties of recovering taenia-type eggs through fecal flotation with the varying sensitivities between float 1 (41%) and float 2 (28%) (C. Adolph et al., 2017; Dryden et al., 2005; Little et al., 2015).

The float/sediment technique in this study performed on the same fecal sample, had higher sensitivity (40%) than flotation 2 (28%), which demonstrated the density of taeniid eggs and the difficulty in recovering them through fecal flotation (Bucur et al., 2019). A similar study in Canada, found that sedimentation had a higher sensitivity (49-53%) when compared to centrifugal sucrose flotation (35-39%) (Kolapo, 2023). The float/sediment technique performed in our study, shows that a flotation technique should not be used alone for the recovery of taenia-type eggs.

Diagnostic sensitivity of fecal PCR used in this study was low, which is different from other reports that show the superiority of fecal PCR when compared to fecal flotation or sedimentation (Kolapo, 2023; Kolapo et al., 2021b; Yasur-Landau et al., 2023). In a study comparing PCR sensitivity between DNA recovered from feces and DNA recovered from taenia-type eggs on flotation, fecal PCR performed better 32/317 (10.1%) to float PCR 19/317 (6%) (Yasur-Landau et al., 2023). This is despite taenia-type eggs being recovered from the same number of samples, 32/317 (10.1%), that were positive on fecal PCR (Yasur-Landau et al., 2023). Ideally, fecal PCR can be used to identify the false positive samples when taenia-type eggs are not found on flotation, sedimentation, and even adult cestode recovery. However, in our study there was no significant difference seen between flotation and sediment when compared to PCR because all of tests performed similarly to another.

Reasons our fecal PCR sensitivity could be low are possibly due to the effect of multiple-freeze thaw cycles of carcasses and samples from collection to testing. Although previous literature supports that freezing taenia-type eggs at -20 and -80ºC does not reduced the sensitivity of fecal flotations (J. Schurer et al., 2013). Additionally, there is an overall reduction in the amount of feces collected due to the presence of hair and/or undigested bone being present, which is a stark difference when compared to the amount of feces present in domestic dogs fed a commercial diet. There is also a link between low worm burdens and fecal inhibitors to reduced sensitivity in PCR (Lahmar et al., 2007).

Diagnostic specificity was better in fecal flotation than fecal sedimentation and PCR when compared to the gold standard of adult egg recovery. We can conclude that if taeniid eggs are recovered in fecal flotation then the animal is most likely infected with taeniid tapeworms. Although, taenia-type eggs cannot be differentiated past the genus level when visualized on fecal flotation, domestic canines that are positive for taenia-type eggs on fecal float in an endemic region should be highly suspicious of *E. multilocularis* infection (Kolapo et al., 2021b). In comparison, the diagnostic specificity for sedimentation and PCR were lower when compared to fecal flotation. Some reasonings for the decreased specificity in sedimentation could be due to ingestion of taenia-type eggs during coprophagy, which leads to taenia-type eggs passing through the gastrointestinal tract (A. M. Zajac et al., 2021). Because all four sedimentation samples were truly negative for taeniid adults, this could be a likely reason. Additionally, the reason for low specificity in fecal PCR could be due to the inability to detect low intensity infections and adults becoming fragile after multiple freeze-thaw cycles thereby making them difficult to identify during intestinal analysis.

Finally, it is notoriously difficulty of extract DNA from taenia-type eggs that have a thick, striated shell built to withstand harsh temperatures and conditions (Klein et al., 2014). Therefore, choosing a reliable DNA extraction method is paramount to the success of any PCR test. In our study the QIAamp® DNA Stool Mini Kit was used for the extraction of DNA from feces with no modifications. The performance of the same kit was compared to the FastDNA™ SPIN Kit for Soil for the detection of taenia-type eggs washed from produce (Frey et al., 2019). Because of difficulty with breaking open taeniid eggs, the kit was used with modifications, incorporating the addition of ASL buffer followed by, 8 freeze-thaw cycles in liquid nitrogen and 95°C water baths, and 20uL of Proteinase K, then the samples were incubated at 56°C for 3 hours (Frey et al., 2019). The Fast DNA™ Spin kit for Soil included bead beating in the extraction step. Even with these modifications added, the QIAamp® DNA Mini Stool kit still underperformed on qPCR with higher Cq values when compared to the FastDNA™ Spin kit for Soil (Frey et al., 2019). Therefore, the addition of bead beating to the extraction method may increase the detection of taeniid DNA in samples. In fact, Kolapo (2023), used the FastDNA™ Spin kit for Soil in the extraction of taeniid DNA from fecal samples, which yielded 92-93% sensitivity on a fecal qPCR with a melting curve analysis (copro-qPCR-MCA) (Kolapo, 2023). Additionally, bead beating has proven to increase DNA isolation in other parasites, such as *Cryptosporidium parvum* and *Giardia duodenalis* and nematodes such as *Trichuris trichura* (Cazeaux et al., 2022; Kaisar et al., 2017).

Our study shows the high percentage of cestode infection in wild canids, especially *Echinococcus multilocularis*. This highlights the importance of wildlife surveillance programs and the need for improved diagnostic strategies in domestic dogs. Especially with the occurrence of *E. multilocularis* spillovers into domestic dogs and humans

4.6 Conclusion

Based on our study, adult taeniid recovery remains the gold standard for the detection of *Echinococcus* spp. in wild canids and domestic dogs. Fecal flotation can be used for the detection of taenia-type eggs in feces but could be paired with a sedimentation for a complete assessment of taenia-type eggs present in a fecal sample. Fecal PCR can be a reliable method for detection of taeniid DNA, however, the ability to break apart taenia-type eggs most notably affects PCR sensitivity. Therefore, choosing a reliable extraction method, like bead beating, may aid in detecting taeniid DNA in fecal samples. The need to improve fecal diagnostics for the detection of taenia-type eggs and taeniid DNA in wild canid and domestic dogs remains.

Chapter 5 - Conclusions and Future Directions

5.1 Conclusions

Tapeworms are ubiquitous, flat, segmented, parasites that are found in a plethora of aquatic and terrestrial hosts. The Diphyllobothriidean and Cyclophyllidean tapeworms of veterinary importance remain a challenge for taxonomic classification, diagnosis and treatment. The use of PCR has aided in eliminating and reclassifying taxa in the genus *Spirometra* and *Mesocestoides.* Also, aiding in the identification of two distinct species, canine and feline, in *Dipylidium caninum* and various haplotypes of *Echinococcus multilocularis* found worldwide. The improvement of molecular testing has provided new insights into tapeworm infections in the United States and have shown that tapeworm infections are more common than previously documented. In fact, many studies have shown that using fecal flotation to diagnose *D. caninum* and taeniid tapeworm infections underestimates the prevalence (C. Adolph et al., 2017; Little et al., 2023). Additionally, fecal flotation, while it remains a reliable diagnostic technique, should not be used alone to diagnose cestode infections (Kolapo et al., 2021b; Little et al., 2023). The development of antigen testing for *D. caninum* infections has increased the number of infections detected in domestic dogs and cats, even when proglottids are not seen in feces and egg packets are not detected on fecal flotation (Elsemore et al., 2023). Despite this, there are challenges that remain for the detection of taeniid tapeworms, and a reliable method for detection has yet to be determined. Taenia-type eggs, like *D. caninum* egg packets, are not only difficult to float, but eggs are shed intermittently, and number of eggs found depends on the intensity of infection. More, importantly, the eggshell of taenia-type eggs is thick and difficult to break open, making taeniid DNA difficult to detect in feces when using molecular tests. Different methods like multiple freeze-thaw cycles in liquid nitrogen and chemical washes to weaken the egg shell have varying results and the process is labor some (Frey et al., 2019; Mathis et al., 1996). Antigen testing for *Echinococcus* spp. has proven to be effective, but the difficulties with detection in low burdens and cross-reactivity with *Taenia* spp. have not been fixed for this to be considered a reliable method. As there is an increase in the number of domestic and wild canids positive for *Echinococcus multilocularis*, the expansion of coyotes into urban areas in the United States, and the high zoonotic health risk that *E. multilocularis* poses to the human and animal health, the need to develop a reliable diagnostic method is important.

Lastly, treatment of tapeworms is achieved through the use of Praziquantel, but the recent reports of Praziquantel resistance to normal and elevated levels in the treatment of *D. caninum* is concerning. Especially since the mechanism of action of how praziquantel acts on tapeworms remains unknown.

In Chapter 2, the prevalence of Dipylidium caninum in flea populations in Florida is likely low with a total of 3.8% of flea pools from cats and the environment positive for *D. caninum*. This matches previous reports of low number of infected fleas when pooled tested (Abdullah et al., 2019; Low et al., 2017). *Ctenocephalides felis* also causes health problems to the infested host causing anemia and flea allergy dermatitis (FAD). The cat flea is also an intermediate host for parasites such as *Dipylidium caninum*, the flea tapeworm, and *Acanthocheilonema reconditum*, a cutaneous filarial nematode. Previous chapters in this thesis work have described that using fecal flotation alone to diagnose *D. caninum* infection underestimates the number of animals infected, but fecal antigen testing has helped to improve the detection of *D. caninum* in infected animals. Alternatively, our work has shown that pooled testing of fleas can also be a reliable method for assessing the infection risk to humans and animals in the environment. It is known that *D. caninum* infection is found in locations where

Ctenocephalides felis, the cat flea intermediate host, is also found, and pooled testing could be used to assess infection risk in situations where fecal antigen testing is not available (Little et al., 2023). In addition, new reports suggest that *D. caninum* is actually two different species, canine and feline, and the feline species of *D. caninum* has a shorter life span and prepatent period when infecting cats versus dogs and the same with the canine species of *D. caninum* (Beugnet et al., 2014, 2018; Low et al., 2017). The discovery of two distinct species will be helpful especially when choosing to use cats or dogs as the definitive host for an establishment of a laboratory colony. However, it is yet to be determined how having to distinct species of *D. caninum* affects treatment with praziquantel since none of the previous cases reported identified the species of *D. caninum* (J. Jesudoss Chelladurai et al., 2018; Loftus et al., 2022; Oehm et al., 2024). Concluding, it is important to determine the role the two species, canine and feline, of *D. caninum* have, if any, in the development of praziquantel resistance. Also, if the seasonality of fleas plays a role in the epidemiology of *D. caninum* species across the United States.

In Chapter 3, the first reports of *Echinococcus multilocularis* in wild canids in Kansas and Missouri. This is also the first report of the European haplotype in wild canids and in Missouri where cases in domestic dogs have been reported (Kuroki et al., 2020, 2022). This is also the highest prevalence to date, 31.4%, in coyotes in the United States (Conlon et al., 2023; Garrett, 2021). My findings document that the European strain of *E. multilocularis* is endemic in the Midwest United States. The challenge is that every endemic region is different, and each region may not have the same factors affecting transmission and endemicity (Hegglin & Deplazes, 2013). But, determining the definitive host responsible for *E. multilocularis* transmission, and the interactions between the intermediate and definitive hosts involved are important baseline information for the implementation of control and prevention programs . In the United States, a baseline of *E. multilocularis* endemic regions is being established through wild canid surveillance and partially through the screening of owned domestic dogs (Conlon et al., 2023; Evason et al., 2025; Garrett, 2021). However, robust surveillance also involves looking at rodent communities; agricultural practices; integration of, and interaction with, wildlife; and density of wild canids and domestic dogs in an endemic region (Giraudoux, 1997; Giraudoux et al., 2002). Therefore, it is important to start an integrated approach to surveillance in order to assess the public health risk for all populations of domestic dogs and humans (Kern, 2004; Piarroux et al., 2013; Wang et al., 2006).

In chapter 4, the sensitivity of centrifugal fecal flotation, fecal sedimentation, and fecal PCR for the detection of taeniid tapeworms in comparison to the gold standard of post-mortem taeniid adult recovery. This work demonstrated that the sensitivity of all three diagnostic techniques was 51% compared to adult taeniid recovery, 69%. I also demonstrated that fecal flotation is not a reliable method to be used alone in the recovery of taenia-type eggs. In addition, the sediment from a float can be analyzed to increase the recovery of taenia-type eggs when using a fecal flotation. Our study did not support the pairing of sedimentation with fecal PCR or fecal flotation with fecal PCR to increase taenia-type egg recovery, despite previous studies demonstrating that fecal PCR is a superior method to sedimentation and flotation (Kolapo, 2023; Yasur-Landau et al., 2023). However, PCR is needed to identify taenia-type eggs to species. The success of fecal PCR depends on how well DNA can be extracted from taenia-type eggs, bead beating thus far has proven to be the superior method for DNA extraction. As seen in Kolapo et al. (2023) when the FastDNA™ Spin Skit for soil demonstrated a dramatic increase in PCR sensitivity, especially when paired with quantitative PCR with a melting curve analysis (Kolapo, 2023). Interestingly, the same multiplex primers designed by Trachsel et al. (2007), and used for conventional PCR described throughout this dissertation, were adapted into quantitative PCR (qPCR) with melting curve analysis protocol used in the aforementioned Kolapo (2023), and Frey et al. (2019). Although not mentioned specifically in our study, the intensity of *Echinococcus* spp. adults found through adult cestode recovery ranged from 2 gravid proglottids to 1 intact adult to numerous immature adults and numerous intact mature adults. Because there is so much variability in the infection intensities, taeniid egg shedding and recovery, and fecal inhibitors in wild and domestic canids, a method with high sensitivity and specificity is needed, especially in large scale surveillance.

5.2 Future Studies

I would like to focus on continuing Echinococcus surveillance in the United States. The surveillance described in this dissertation highlights the endemicity of European haplotypes in North America. The reports of echinococcosis in domestic canines in states like Colorado and Kansas, plus increasing anecdotal reports of echinococcosis and alveolar echinococcosis in Kansas, prove that surveillance is needed. First, I want to establish the baseline transmission cycle present in endemic regions where positive coyotes have been collected. I would like to collect rodents for surveillance of alveolar hydatid cysts in the liver, and screen feces of domestic dogs used for hunting, in shelters, or from owned pets that predate on rodents. Establishing a baseline transmission cycle helps to identify if domestic dogs or a wild canid are the main contributor of eggs in the environment therefore, a prevention and control strategy can be developed to slow the infection rate in other domestic dogs and humans. I would like to evaluate the copro-qPCR-MCA method on the coyote feces collected during this project to evaluate if that method will improve the copro-PCR sensitivity in the fecal samples collected during this project. Disease surveillance in coyotes is important especially as more coyote populations adapt to urban settings and interact with domestic animals and humans. I would like to also survey coyotes and coyote scat for additional parasites that are being transported, and introduced, into new areas. I would like to continue working with the trapping community to promote awareness of zoonotic parasites that can be transmitted in the species targeted for fur and nuisance.

References

Abdullah, S., Helps, C., Tasker, S., Newbury, H., & Wall, R. (2019). Pathogens in fleas collected from cats and dogs: Distribution and prevalence in the UK. *Parasites & Vectors*, *12*(1), 71. https://doi.org/10.1186/s13071-019-3326-x

Adolph, C. B., & Peregrine, A. S. (2021). Tapeworms. In *Greene's Infectious Diseases of the Dog and Cat* (pp. 1455–1484). Elsevier. https://doi.org/10.1016/B978-0-323-50934-3.00115-4

Adolph, C., Barnett, S., Beall, M., Drake, J., Elsemore, D., Thomas, J., & Little, S. (2017). Diagnostic strategies to reveal covert infections with intestinal helminths in dogs. *Veterinary Parasitology*, *247*, 108–112. https://doi.org/10.1016/j.vetpar.2017.10.002

Allan, J. C., Craig, P. S., Garcia Noval, J., Mencos, F., Liu, D., Wang, Y., Wen, H., Zhou, P., Stringer, R., Rogan, M., & Zeyhle, E. (1992). Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. *Parasitology*, *104*(2), 347–355. https://doi.org/10.1017/S0031182000061801

AlSalman, A., Mathewson, A., Martin, I. W., Mahatanan, R., & Talbot, E. A. (2023). Cystic Echinococcosis in Northern New Hampshire, USA. *Emerging Infectious Diseases*, *29*(5). https://doi.org/10.3201/eid2905.221828

Alvarez Rojas, C. A., Kronenberg, P. A., Aitbaev, S., Omorov, R. A., Abdykerimov, K. K., Paternoster, G., Müllhaupt, B., Torgerson, P., & Deplazes, P. (2020). Genetic diversity of Echinococcus multilocularis and Echinococcus granulosus sensu lato in Kyrgyzstan: The A2 haplotype of E. multilocularis is the predominant variant infecting humans. *PLOS Neglected Tropical Diseases*, *14*(5), e0008242. https://doi.org/10.1371/journal.pntd.0008242

Andersen, K. (1972). *Studies of the helminth fauna of Norway XXI: The influence of population size (intensity of infection) on morphological characters in Diphyllobothrium dendriticum Nitzsch in the golden hamster (Mesocricetus auratus Waterhouse)*. *20*(1), 1–7.

Arizono, N., Shedko, M., Yamada, M., Uchikawa, R., Tegoshi, T., Takeda, K., & Hashimoto, K. (2009). Mitochondrial DNA divergence in populations of the tapeworm Diphyllobothrium nihonkaiense and its phylogenetic relationship with Diphyllobothrium klebanovskii. *Parasitology International*, *58*(1), 22–28. https://doi.org/10.1016/j.parint.2008.09.001

Armua-Fernandez, M. T., Castro, O. F., Crampet, A., Bartzabal, Á., Hofmann-Lehmann, R., Grimm, F., & Deplazes, P. (2014). First case of peritoneal cystic echinococcosis in a domestic cat caused by Echinococcus granulosus sensu stricto (genotype 1) associated to feline immunodeficiency virus infection. *Parasitology International*, *63*(2), 300–302. https://doi.org/10.1016/j.parint.2013.11.005

Baden, L. R., & Elliott, D. D. (2003). Case 4-2003: A 42-Year-Old Woman with Cough, Fever, and Abnormalities on Thoracoabdominal Computed Tomography. *New England Journal of Medicine*, *348*(5), 447–455. https://doi.org/10.1056/NEJMcpc020027

Ballard, N. B., & Vande Vusse, F. J. (1983). Echinococcus multilocularis in Illinois and Nebraska. *The American Society of Parasitologists*, *69*(4), 790–791.

Beaver, P. C. (1954). Parasitic diseases of animals and their relation to public health. *Veterinary Medicine*, *49*(5), 199–205.

Beugnet, F., Labuschagne, M., Fourie, J., Jacques, G., Farkas, R., Cozma, V., Halos, L., Hellmann, K., Knaus, M., & Rehbein, S. (2014). Occurrence of Dipylidium caninum in fleas from client-owned cats and dogs in Europe using a new PCR detection assay. *Veterinary Parasitology*, *205*(1–2), 300–306. https://doi.org/10.1016/j.vetpar.2014.06.008

Beugnet, F., Labuschagne, M., Vos, C. D., Crafford, D., & Fourie, J. (2018). Analysis of *Dipylidium caninum* tapeworms from dogs and cats, or their respective fleas: Part 2. Distinct canine and feline host association with two different *Dipylidium caninum* genotypes. *Parasite*, *25*, 31. https://doi.org/10.1051/parasite/2018029

Beveridge, I., Friend, S., Jeganathan, N., & Charles, J. (1998). Proliferative sparganosis in Australian dogs. *Australian Veterinary Journal*, *76*(11), 757–759. https://doi.org/10.1111/j.1751-0813.1998.tb12309.x

Blatz, A. M., Laycock, K. M., Kaur, I., & Swami, S. K. (2022). Acute Respiratory Failure With Hemoptysis in a Teenager Due to Cystic Echinococcosis. *Journal of the Pediatric Infectious Diseases Society*, *11*(1), 33–35. https://doi.org/10.1093/jpids/piab086

Bonelli, P., Masu, G., Dei Giudici, S., Pintus, D., Peruzzu, A., Piseddu, T., Santucciu, C., Cossu, A., Demurtas, N., & Masala, G. (2018). Cystic echinococcosis in a domestic cat (*Felis catus*) in Italy. *Parasite*, *25*, 25. https://doi.org/10.1051/parasite/2018027

Bouchard, É., Schurer, J. M., Kolapo, T., Wagner, B., Massé, A., Locke, S. A., Leighton, P., & Jenkins, E. J. (2021). Host and geographic differences in prevalence and diversity of gastrointestinal helminths of foxes (Vulpes vulpes), coyotes (Canis latrans) and wolves (Canis lupus) in Québec, Canada. *International Journal for Parasitology: Parasites and Wildlife*, *16*, 126–137. https://doi.org/10.1016/j.ijppaw.2021.09.002

Bourguinat, C., Lee, A. C. Y., Lizundia, R., Blagburn, B. L., Liotta, J. L., Kraus, M. S., Keller, K., Epe, C., Letourneau, L., Kleinman, C. L., Paterson, T., Gomez, E. C., Montoya-Alonso, J. A., Smith, H., Bhan, A., Peregrine, A. S., Carmichael, J., Drake, J., Schenker, R., … Prichard, R. K. (2015). Macrocyclic lactone resistance in Dirofilaria immitis: Failure of heartworm preventives and investigation of genetic markers for resistance. *Veterinary Parasitology*, *210*(3–4), 167–178. https://doi.org/10.1016/j.vetpar.2015.04.002

Bowman, D. D. (2021). *Georgis' parasitology for veterinarians* (Eleventh edition). Elsevier.

Bowman, D. D., Lin, D.-S., Johnson, R. C., Lynn, R. C., Hepler, D. I., & Stansfield, D. G. (1991). Effects of nitroscanate on adult Taenia pisiformis in dogs with experimentally induced infections. *American Journal of Veterinary Research*, *52*(9), 1542–1544. https://doi.org/10.2460/ajvr.1991.52.09.1542

Boyce, W., Shender, L., Schultz, L., Vickers, W., Johnson, C., Ziccardi, M., Beckett, L., Padgett, K., Crosbie, P., & Sykes, J. (2011). Survival analysis of dogs diagnosed with canine peritoneal larval cestodiasis (Mesocestoides spp.). *Veterinary Parasitology*, *180*(3–4), 256–261. https://doi.org/10.1016/j.vetpar.2011.03.023

Braae, U. C., Thomas, L. F., Robertson, L. J., Dermauw, V., Dorny, P., Willingham, A. L., Saratsis, A., & Devleesschauwer, B. (2018). Epidemiology of Taenia saginata taeniosis/cysticercosis: A systematic review of the distribution in the Americas. *Parasites & Vectors*, *11*(1), 518. https://doi.org/10.1186/s13071-018-3079-y

Brabec, J., Uribe, M., Chaparro-Gutiérrez, J. J., & Hermosilla, C. (2022). Presence of *Spirometra mansoni* , Causative Agent of Sparganosis, in South America. *Emerging Infectious Diseases*, *28*(11), 2347–2350. https://doi.org/10.3201/eid2811.220529

Brandell, E. E., Jackson, M. K., Cross, P. C., Piaggio, A. J., Taylor, D. R., Smith, D. W., Boufana, B., Stahler, D. R., & Hudson, P. J. (2022). Evaluating noninvasive methods for estimating cestode prevalence in a wild carnivore population. *PLOS ONE*, *17*(11), e0277420. https://doi.org/10.1371/journal.pone.0277420

Broglia, A., & Kapel, C. (2011). Changing dietary habits in a changing world: Emerging drivers for the transmission of foodborne parasitic zoonoses. *Veterinary Parasitology*, *182*(1), 2–13. https://doi.org/10.1016/j.vetpar.2011.07.011

Bryan, R. T., & Schantz, P. M. (1989). Echinococcosis (hydatid disease). *Journal of the American Veterinary Medical Association*, *195*(9), 1214–1217.

Bucur, I., Gabriël, S., Van Damme, I., Dorny, P., & Vang Johansen, M. (2019). Survival of Taenia saginata eggs under different environmental conditions. *Veterinary Parasitology*, *266*, 88–95. https://doi.org/10.1016/j.vetpar.2018.12.011

Buergelt, C. D., Greiner, E. C., & Senior, D. F. (1984). Proliferative Sparganosis in a Cat. *The Journal of Parasitology*, *70*(1), 121. https://doi.org/10.2307/3281933

Burgu, A., Sarimehmetogğlu, O., & Vural, S. A. (2004). Cystic echinococcosis in a stray cat. *Veterinary Record*, *155*(22), 711–712. https://doi.org/10.1136/vr.155.22.711

Burlew, B. P., Cook, E. W., & Thiele, J. S. (1990). Asymptomatic Pulmonary Cyst in a College Student. *Chest*, *98*(2), 455–457. https://doi.org/10.1378/chest.98.2.455

Cabot, R. C., Scully, R. E., Mark, E. J., McNeely, W. F., McNeely, B. U., Weller, P. F., & Moskowitz, G. (1987). Case 45-1987: A 16-Year-Old Girl with Hepatic and Pulmonary Masses after a Sojourn in Bolivia. *New England Journal of Medicine*, *317*(19), 1209–1218. https://doi.org/10.1056/NEJM198711053171908

CAPC. (2025, February 28). *Dipylidium caninum*. Companion Animal Parasite Council (CAPC). https://capcvet.org/guidelines/dipylidium-caninum/

Cardona, G. A., & Carmena, D. (2013). A review of the global prevalence, molecular epidemiology and economics of cystic echinococcosis in production animals. *Veterinary Parasitology*, *192*(1–3), 10–32. https://doi.org/10.1016/j.vetpar.2012.09.027

Carta, S., Corda, A., Tamponi, C., Dessì, G., Nonnis, F., Tilocca, L., Cotza, A., Knoll, S., Varcasia, A., & Scala, A. (2021). Clinical forms of peritoneal larval cestodiasis by Mesocestoides spp. in dogs: Diagnosis, treatment and long term follow-up. *Parasitology Research*, *120*(5), 1727–1735. https://doi.org/10.1007/s00436-021-07107-w

Carter, C., Bonatti, H., Hranjec, T., Barroso, L. F., Donowitz, G., Sawyer, R. G., & Schirmer, B. (2009). Epigastric Cystic Echinococcus Involving Stomach, Liver, Diaphragm, and Spleen in an Immigrant from Afghanistan. *Surgical Infections*, *10*(5), 453–456. https://doi.org/10.1089/sur.2008.051

Castrodale, L. J., Beller, M., Wilson, J. F., Schantz, P. M., McManus, D. P., Zhang, L.-H., Fallico, F. G., & Sacco, F. D. (2002). Two atypical cases of cystic echinococcosis (Echinococcus granulosus) in Alaska, 1999. *The American Journal of Tropical Medicine and Hygiene*, *66*(3), 325–327. https://doi.org/10.4269/ajtmh.2002.66.325

Catalano, S., Lejeune, M., Liccioli, S., Verocai, G. G., Gesy, K. M., Jenkins, E. J., Kutz, S. J., Fuentealba, C., Duignan, P. J., & Massolo, A. (2012). *Echinococcus multilocularis* in Urban Coyotes, Alberta, Canada. *Emerging Infectious Diseases*, *18*(10), 1625–1628. https://doi.org/10.3201/eid.1810.120119

Cazeaux, C., Lalle, M., Durand, L., Aubert, D., Favennec, L., Dubey, J. P., Geffard, A., Villena, I., & La Carbona, S. (2022). Evaluation of real-time qPCR-based methods to detect the DNA of the three protozoan parasites Cryptosporidium parvum, Giardia duodenalis and Toxoplasma gondii in the tissue and hemolymph of blue mussels (M. edulis). *Food Microbiology*, *102*, 103870. https://doi.org/10.1016/j.fm.2021.103870

CDC. (2017, December 30). *Sparganosis*. DPDx - Laboratory Identification of Parasites of Public Health Importance. https://www.cdc.gov/dpdx/sparganosis/index.html

CDC. (2019a, May 14). *Dyphyllobothriasis*. DPDx - Laboratory Identification of Parasites of Public Health Importance. https://www.cdc.gov/dpdx/diphyllobothriasis/index.html

CDC. (2019b, July 10). *Dipylidium caninum*. DPDx - Laboratory Identification of Parasites of Public Health Importance. https://www.cdc.gov/dpdx/dipylidium/

CDC. (2019c, July 15). *Echinococcosis*. DPDx - Laboratory Identification of Parasites of Public Health Importance. https://www.cdc.gov/dpdx/echinococcosis/index.html

CDC. (2019d, September 23). *Mesocestoidiasis*. DPDx - Laboratory Identification of Parasites of Public Health Importance. https://www.cdc.gov/dpdx/mesocestoidiasis/index.html

CDC. (2019e, September 23). *Mesocestoidiasis*. Laboratory Identification of Parasites of Public Health Concern - Center for Disease Control (CDC). https://www.cdc.gov/dpdx/mesocestoidiasis/index.html

Cerda, J. R., Buttke, D. E., & Ballweber, L. R. (2018). *Echinococcus* spp. Tapeworms in North America. *Emerging Infectious Diseases*, *24*(2), 230–235. https://doi.org/10.3201/eid2402.161126

Cho, S.-H., Kim, T.-S., Kong, Y., Na, B.-K., & Sohn, W.-M. (2013). Tetrathyridia of Mesocestoides lineatus in Chinese Snakes and Their Adults Recovered from Experimental Animals. *The Korean Journal of Parasitology*, *51*(5), 531–536. https://doi.org/10.3347/kjp.2013.51.5.531

Coles, G. C. (1979). The effect of Praziquantel on *Schistosoma mansoni*. *Journal of Helminthology*, *53*(1), 31–33. https://doi.org/10.1017/S0022149X00005691

Colorado Conservation Alliance Inc. (2025, January 13). *Colorado Parks and Wildlife (CPW) Begins Capture and Transport of British Colombia Wolves to Colorado 24 Hours after Announcing Canadian Wolf Capture Operations Have Commenced* [News]. PR Newswire. https://www.prnewswire.com/news-releases/colorado-parks-and-wildlife-cpw-begins-capture-and-transport-of-british-colombia-wolves-to-colorado-24-hours-after-announcing-canadian-wolf-capture-operations-have-commenced-302349421.html

Conboy, G. (2009). Cestodes of Dogs and Cats in North America. *Veterinary Clinics of North America: Small Animal Practice*, *39*(6), 1075–1090. https://doi.org/10.1016/j.cvsm.2009.06.005

Conlon, C. L., Schuler, K. L., Lejeune, M., & Whipps, C. M. (2023). Novel Report of the European Variant of Echinococcus multilocularis in Coyotes (Canis latrans) in New York State. *Journal of Parasitology*, *109*(4). https://doi.org/10.1645/22-104

Conn, D. B. (1990). The rarity of asexual reproduction among Mesocestoides tetrathyridia (Cestoda). *The Journal of Parasitology*, *76*(3), 453–455.

Conn, D. B., Galán-Puchades, M.-T., & Fuentes, M. V. (2011). Normal and Aberrant Mesocestoides Tetrathyridia from Crocidura spp. (Soricimorpha) in Corsica and Spain. *Journal of Parasitology*, *97*(5), 915–919. https://doi.org/10.1645/GE-2441.1

Conraths, F. J., & Deplazes, P. (2015). Echinococcus multilocularis: Epidemiology, surveillance and state-of-the-art diagnostics from a veterinary public health perspective. *Veterinary Parasitology*, *213*(3–4), 149–161. https://doi.org/10.1016/j.vetpar.2015.07.027

Cornish, R. A., & Bryant, C. (1976). Changes in energy metabolism due to anthelmintics in Fasciola hepatica maintained in vitro. *International Journal for Parasitology*, *6*(5), 393–398. https://doi.org/10.1016/0020-7519(76)90024-2

Craig, P., Mastin, A., Van Kesteren, F., & Boufana, B. (2015). Echinococcus granulosus: Epidemiology and state-of-the-art of diagnostics in animals. *Veterinary Parasitology*, *213*(3–4), 132–148. https://doi.org/10.1016/j.vetpar.2015.07.028

Craig, P. S., Hegglin, D., Lightowlers, M. W., Torgerson, P. R., & Wang, Q. (2017). Echinococcosis. In *Advances in Parasitology* (Vol. 96, pp. 55–158). Elsevier. https://doi.org/10.1016/bs.apar.2016.09.002

Crellin, J. R., & Harmon, W. M. (1980). Cestodes of the coyote (Canis latrans) in San Joaquin Valley, California. *The Journal of Parasitology*, *66*(1), 180–181.

Crosbie, P. R., Boyce, W. M., Platzer, E. G., Nadler, S. A., & Kerner, C. (1998). Diagnostic procedures and treatment of eleven dogs with peritoneal infections caused by Mesocestoides spp. *Journal of the American Veterinary Medical Association*, *213*(11), 1578–1583, 1570.

Dahlem, D., Bangoura, B., Ludewig, E., Glowienka, N., Baldauf, K., Stoeckel, F., & Burgener, I. (2015). Tetrathyridiosis in a domestic shorthair cat. *Journal of Feline Medicine and Surgery Open Reports*, *1*(2), 2055116915615595. https://doi.org/10.1177/2055116915615595

David, E. D., & Lindquist, W. D. (1982). Determination of the specific gravity of certain helminth eggs using sucrose density gradient centrifugation. *The Journal of Parasitology*, *68*(5), 916–919.

Dayan, A. D. (2003). Albendazole, mebendazole and praziquantel. Review of non-clinical toxicity and pharmacokinetics. *Acta Tropica*, *86*(2–3), 141–159. https://doi.org/10.1016/S0001-706X(03)00031-7

De La Cruz-Saldana, T., Bustos, J. A., Requena-Herrera, M. P., Martinez-Merizalde, N., Ortiz-Cam, L., Cáceres, A. L., Guzman, C., Gavidia, C. M., Ugarte-Gil, C., & Castillo-Neyra, R. (2024). *A scoping review on control strategies for* Echinococcus granulosus sensu lato. Infectious Diseases (except HIV/AIDS). https://doi.org/10.1101/2024.08.21.24312335

De Lima, N. F., Picanço, G. D. A., Valencia, D. G. R., Villegas, E. O. L., Mellado, M. D. R. E., Ambrosio, J. R., & Vinaud, M. C. (2021). Alterations in Taenia crassiceps cysticerci cytoskeleton induced by nitazoxanide and flubendazole. *Acta Tropica*, *221*, 106027. https://doi.org/10.1016/j.actatropica.2021.106027

Dell, B., Newman, S. J., Purple, K., Miller, B., Ramsay, E., Donnell, R., & Gerhold, R. W. (2020). Retrospective investigation of Echinococcus canadensis emergence in translocated elk (Cervus canadensis) in Tennessee, USA, and examination of canid definitive hosts. *Parasites & Vectors*, *13*(1), 330. https://doi.org/10.1186/s13071-020-04198-9

Demos, N. J. (1974). Echinococcus cyst of the liver in New Jersey: Treated by left hepatic lobectomy. *The Journal of the Medical Society of New Jersey*, *71*(11), 859–862.

Deplazes, P., Alther, P., Tanner, I., Thompson, R. C., & Eckert, J. (1999). Echinococcus multilocularis coproantigen detection by enzyme-linked immunosorbent assay in fox, dog, and cat populations. *The Journal of Parasitology*, *85*(1), 115–121.

Deplazes, P., Eichenberger, R. M., & Grimm, F. (2019). Wildlife-transmitted Taenia and Versteria cysticercosis and coenurosis in humans and other primates. *International Journal for Parasitology: Parasites and Wildlife*, *9*, 342–358. https://doi.org/10.1016/j.ijppaw.2019.03.013

Deplazes, P., Gottstein, B., Eckert, J., Jenkins, D. J., Ewald, D., & Jimenez-Palacios, S. (1992). Detection ofEchinococcus coproantigens by enzyme-linked immunosorbent assay in dogs, dingoes and foxes. *Parasitology Research*, *78*(4), 303–308. https://doi.org/10.1007/BF00937088

Deplazes, P., Rinaldi, L., Alvarez Rojas, C. A., Torgerson, P. R., Harandi, M. F., Romig, T., Antolova, D., Schurer, J. M., Lahmar, S., Cringoli, G., Magambo, J., Thompson, R. C. A., & Jenkins, E. J. (2017). Global Distribution of Alveolar and Cystic Echinococcosis. In *Advances in Parasitology* (Vol. 95, pp. 315–493). Elsevier. https://doi.org/10.1016/bs.apar.2016.11.001

Drake, D. A., Carreño, A. D., Blagburn, B. L., Little, S. E., West, M. D., Hendrix, C. M., & Johnson, C. M. (2008). Proliferative sparganosis in a dog. *Journal of the American Veterinary Medical Association*, *233*(11), 1756–1760. https://doi.org/10.2460/javma.233.11.1756

Dryden, M. W., Boyer, J. E., & Smith, V. (1994). Techniques for Estimating On-Animal Populations of Ctenocephalides felis (Siphonaptera: Pulicidae). *Journal of Medical Entomology*, *31*(4), 631–634. https://doi.org/10.1093/jmedent/31.4.631

Dryden, M. W., & Broce, A. B. (1993). Development of a Trap for Collecting Newly Emerged Ctenocephalides felis (Siphonaptera: Pulicidae) in Homes. *Journal of Medical Entomology*, *30*(5), 901–906. https://doi.org/10.1093/jmedent/30.5.901

Dryden, M. W., Payne, P. A., Ridley, R., & Smith, V. (2005). Comparison of Common Fecal Flotation Techniques for the Recovery of Parasite Eggs and Oocysts. *Veterinary Therapeutics*, *6*(1).

Duscher, G., Prosl, H., & Joachim, A. (2005). Scraping or shaking?a comparison of methods for the quantitative determination of Echinococcus multilocularis in fox intestines. *Parasitology Research*, *95*(1), 40–42. https://doi.org/10.1007/s00436-004-1260-z

Dyer, W. G., & Klimstra, W. D. (1980). *A Survey of Grey Foxes (Urocyon Cinereoargenteus) for Echinococcus multilocularis in Southern Illinois*. 72–74.

Dyer, W. G., & Klimstra, W. D. (1981). *A Survey of Red Foxes (Vulpes vulpes) for Echinococcus multilocularis*. 133–135.

Eckert, J. (2003). Predictive values and quality control of techniques for the diagnosis of Echinococcus multilocularis in definitive hosts. *Acta Tropica*, *85*(2), 157–163. https://doi.org/10.1016/S0001-706X(02)00216-4

Eckert, J., Conraths, F. J., & Tackmann, K. (2000). Echinococcosis: An emerging or re-emerging zoonosis? *International Journal for Parasitology*, *30*(12–13), 1283–1294. https://doi.org/10.1016/S0020-7519(00)00130-2

Eckert, J., & Deplazes, P. (2004). Biological, Epidemiological, and Clinical Aspects of Echinococcosis, a Zoonosis of Increasing Concern. *Clinical Microbiology Reviews*, *17*(1), 107–135. https://doi.org/10.1128/CMR.17.1.107-135.2004

Eckert, J., Weltgesundheitsorganisation, & International Office of Epizootics (Eds.). (2001). *WHO/OIE manual on Echinococcosis in humans and animals: A public health problem of global concern*. World Organisation for Animal Health.

Edney, J. M. (1940). The occurrence of Echinococcus granulosus in dogs collected in Murfreesboro, Tennessee. *Journal of the Tennessee Academy of Science, 15,* 395.

Edney, J. M. (1949). Echinococcus granulosus in Kentucky dogs. *Journal. Tennessee Academy of Science*, *24*(3), 227.

Elsemore, D., Bezold, T., Geng, J., Hanna, R., Tyrrell, P., & Beall, M. (2023). Immunoassay for detection of *Dipylidium caninum* coproantigen in dogs and cats. *Journal of Veterinary Diagnostic Investigation*, *35*(6), 671–678. https://doi.org/10.1177/10406387231189193

Evans, J. D., Aronstein, K., Chen, Y. P., Hetru, C., Imler, J.-L., Jiang, H., Kanost, M., Thompson, G. J., Zou, Z., & Hultmark, D. (2006). Immune pathways and defence mechanisms in honey bees Apis mellifera. *Insect Molecular Biology*, *15*(5), 645–656. https://doi.org/10.1111/j.1365-2583.2006.00682.x

Evason, M. D., Peregrine, A. S., Jenkins, E. J., Lozoya, C. E., Rund, L. L., Weese, J. S., Castro, P. D. J., & Leutenegger, C. M. (2025). Emerging Echinococcus tapeworms: Fecal PCR detection of Echinococcus multilocularis in 26 dogs from the United States and Canada (2022–2024). *Journal of the American Veterinary Medical Association*, *263*(2), 1–5. https://doi.org/10.2460/javma.24.07.0471

Finck, P. A., & Hunninen, A. V. (1954). Two Human Cases Infected with Echinococcus Cysts, Indigenous from the United States. *The Journal of Parasitology, 40*(6), 706. https://doi.org/10.2307/3273727

Foreyt, W. J., Drew, M. L., Atkinson, M., & McCauley, D. (2009). Echinococcus granulosus in Gray Wolves and Ungulates in Idaho and Montana, USA. *Journal of Wildlife Diseases, 45*(4), 1208–1212. https://doi.org/10.7589/0090-3558-45.4.1208

Franklin, M. A., & Ward, J. W. (1953). Echinococcus Infection in Mississippi. A New Record of a Natural Infection in Dogs. *The Journal of Parasitology*, *39*(5), 574. https://doi.org/10.2307/3273871

French, S. K., Jajou, S., Campbell, G. D., Cai, H. Y., Kotwa, J. D., Peregrine, A. S., & Jardine, C. M. (2018). Echinococcus multilocularis in a wild free-living eastern chipmunk (Tamias striatus) in Southern Ontario: A case report and subsequent field study of wild small mammals. *Veterinary Parasitology: Regional Studies and Reports*, *13*, 234–237. https://doi.org/10.1016/j.vprsr.2018.06.009

Frey, C. F., Jenkins, E., & Lundström-Stadelmann, B. (2019). Editorial overview: From farms and forests to forks? A review of diagnosis and management of globally important zoonotic Echinococcus spp. cestodes. *Food and Waterborne Parasitology*, *16*, e00061. https://doi.org/10.1016/j.fawpar.2019.e00061

Frey, C. F., Marreros, N., Renneker, S., Schmidt, L., Sager, H., Hentrich, B., Milesi, S., & Gottstein, B. (2017). Dogs as victims of their own worms: Serodiagnosis of canine alveolar echinococcosis. *Parasites & Vectors*, *10*(1), 422. https://doi.org/10.1186/s13071-017-2369-0

Fuentes, M. V., GaláN-Puchades, M. T., & Malone, J. B. (2003). Short Report: A new case of Human Mesocestoides infection in the United States. *The American Journal of Tropical Medicine and Hygiene*, *68*(5), 566–567. https://doi.org/10.4269/ajtmh.2003.68.566

Galbreath, K. E., Hoberg, E. P., Cook, J. A., Armién, B., Bell, K. C., Campbell, M. L., Dunnum, J. L., Dursahinhan, A. T., Eckerlin, R. P., Gardner, S. L., Greiman, S. E., Henttonen, H., Jiménez, F. A., Koehler, A. V. A., Nyamsuren, B., Tkach, V. V., Torres-Pérez, F., Tsvetkova, A., & Hope, A. G. (2019). Building an integrated infrastructure for exploring biodiversity: Field collections and archives of mammals and parasites. *Journal of Mammalogy*, *100*(2), 382–393. https://doi.org/10.1093/jmammal/gyz048

Gamble, W. G. (1979). Alveolar hydatid disease in Minnesota. First human case acquired in the contiguous United States. *JAMA: The Journal of the American Medical Association*, *241*(9), 904–907. https://doi.org/10.1001/jama.241.9.904

Garrett, K. (2021, June 21). *Surveillance for Echinococcus species infections among wild canids in Pennsylvania.* [PowerPoint]. AAVP 66th Annual Meeting 2021, Lexington, Kentucky.

Gemmel, M. A., Johnstone, P. D., & Oudemans, G. (1977). The effect of micronised nitroscanate on Echinococcus granulosus and Taenia hydatigena infections in dogs. *Research in Veterinary Science*, *22*(3), 391–392.

Gemmell, M. A., & Oudemans, G. (1975). The effect of nitroscanate on Echinococcus granulosus and Taenia hydatigena infections in dogs. *Research in Veterinary Science*, *19*(2), 217–219.

Gesy, K., Hill, J. E., Schwantje, H., Liccioli, S., & Jenkins, E. J. (2013). Establishment of a European-type strain of *Echinococcus multilocularis* in Canadian wildlife. *Parasitology*, *140*(9), 1133–1137. https://doi.org/10.1017/S0031182013000607

Gesy, K. M., & Jenkins, E. J. (2015). Introduced and Native Haplotypes of *Echinococcus multilocularis* in Wildlife in Saskatchewan, Canada. *Journal of Wildlife Diseases*, *51*(3), 743–748. https://doi.org/10.7589/2014-08-214

Gesy, K. M., Schurer, J. M., Massolo, A., Liccioli, S., Elkin, B. T., Alisauskas, R., & Jenkins, E. J. (2014). Unexpected diversity of the cestode Echinococcus multilocularis in wildlife in Canada. *International Journal for Parasitology: Parasites and Wildlife*, *3*(2), 81–87. https://doi.org/10.1016/j.ijppaw.2014.03.002

Gesy, K., Pawlik, M., Kapronczai, L., Wagner, B., Elkin, B., Schwantje, H., & Jenkins, E. (2013). An improved method for the extraction and quantification of adult Echinococcus from wildlife definitive hosts. *Parasitology Research*, *112*(5), 2075–2078. https://doi.org/10.1007/s00436-013-3371-x

Giraudoux, P. (1997). Population dynamics of fossorial water vole (Arvicola terrestris scherman): A land use and landscape perspective. *Agriculture, Ecosystems & Environment*, *66*(1), 47–60. https://doi.org/10.1016/S0167-8809(97)80706-2

Giraudoux, P., Delattre, P., Takahashi, K., Raoul, F., Quéré, J. P., Craig, P., & Vuitton, D. (2002). *Transmission ecology of Echinococcus multilocularis in wildlife: What can be learned from comparative studies and multiscale approaches?* (pp. 251–266). IOS Press.

Grüner, B., Kern, P., Mayer, B., Gräter, T., Hillenbrand, A., Barth, T. E. F., Muche, R., Henne-Bruns, D., Kratzer, W., & Kern, P. (2017). Comprehensive diagnosis and treatment of alveolar echinococcosis: A single-center, long-term observational study of 312 patients in Germany. *GMS Infectious Diseases*, *5*, Doc01. https://doi.org/10.3205/id000027

Gustinelli, A., Menconi, V., Prearo, M., Caffara, M., Righetti, M., Scanzio, T., Raglio, A., & Fioravanti, M. L. (2016). Prevalence of Diphyllobothrium latum (Cestoda: Diphyllobothriidae) plerocercoids in fish species from four Italian lakes and risk for the consumers. *International Journal of Food Microbiology*, *235*, 109–112. https://doi.org/10.1016/j.ijfoodmicro.2016.06.033

Hall, T. A. (1999). BioEdit: A user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Oxford University Press*, 95–98.

Hall, T. A. (2011). BioEdit: An important software for molecular biology. *GERF Bulletin of Biosciences*, *20*(1), 60–61.

Hammerschmidt, K., & Kurtz, J. (2007). Schistocephalus solidus: Establishment of tapeworms in sticklebacks – fast food or fast lane? *Experimental Parasitology*, *116*(2), 142–149. https://doi.org/10.1016/j.exppara.2006.12.013

Harper, E. P., Oring, J., Powers, H., Sherman, C. E., Wilke, B., Hata, J., Nassar, A., Mendez, J. C., & Libertin, C. R. (2020). 317. Case Series of Echinococcus Infections at Mayo Clinic Florida. *Open Forum Infectious Diseases*, *7*(Supplement_1), S230–S231. https://doi.org/10.1093/ofid/ofaa439.513

Hatsushika, R., Maejima, J., & Kamo, H. (1981). *Experimental studies on the development of Diphyllobothrium macroovatum Jurachno, 1973 from the minke whale, Balaenoptera acutorostrata. II. Experimental infection of the coracidia to marine copepods. 30*(5), 417–427.

Hegglin, D., & Deplazes, P. (2013). Control of Echinococcus multilocularis: Strategies, feasibility and cost–benefit analyses. *International Journal for Parasitology*, *43*(5), 327–337. https://doi.org/10.1016/j.ijpara.2012.11.013

Heidari, Z., Sharbatkhori, M., Mobedi, I., Mirhendi, S. H., Nikmanesh, B., Sharifdini, M., Mohebali, M., Zarei, Z., Arzamani, K., & Kia, E. B. (2019). Echinococcus multilocularis and Echinococcus granulosus in canines in North-Khorasan Province, northeastern Iran, identified using morphology and genetic characterization of mitochondrial DNA. *Parasites & Vectors*, *12*(1), 606. https://doi.org/10.1186/s13071-019-3859-z

Hernandez-Trujillo, H. S., Dalberg, T., Feder, H., & Smith, S. R. (2009). A Fever of Unknown Origin Workup in the Emergency Department Reveals an Unusual Pathogen: *Pediatric Emergency Care*, *25*(10), 684–686. https://doi.org/10.1097/PEC.0b013e3181bec8df

Hildreth, M. B., Sriram, S., Gottstein, B., Wilson, M., & Schantz, P. M. (2000). FAILURE TO IDENTIFY ALVEOLAR ECHINOCOCCOSIS IN TRAPPERS FROM SOUTH DAKOTA IN SPITE OF HIGH PREVALENCE OF *ECHINOCOCCUS MULTILOCULARIS* IN WILD CANIDS. *Journal of Parasitology*, *86*(1), 75–77. https://doi.org/10.1645/0022-3395(2000)086[0075:FTIAEI]2.0.CO;2

Hoang, D. T., Chernomor, O., Von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2018). UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Molecular Biology and Evolution*, *35*(2), 518–522. https://doi.org/10.1093/molbev/msx281

Hochberg, N. S., & Bhadelia, N. (2015). Infections Associated with Exotic Cuisine: The Dangers of Delicacies. *Microbiology Spectrum*, *3*(5), 3.5.01. https://doi.org/10.1128/microbiolspec.IOL5-0010-2015

Hoggard, K. R., Jarriel, D. M., Bevelock, T. J., & Verocai, G. G. (2019). Prevalence survey of gastrointestinal and respiratory parasites of shelter cats in northeastern Georgia, USA. *Veterinary Parasitology: Regional Studies and Reports*, *16*, 100270. https://doi.org/10.1016/j.vprsr.2019.100270

Huss, B. T., Miller, M. A., Corwin, R. M., Hoberg, E. P., & O'Brien, D. P. (1994). Fatal cerebral coenurosis in a cat. *Journal of the American Veterinary Medical Association*, *205*(1), 69–71.

Ing, M. B., Schantz, P. M., & Turner, J. A. (1998). Human Coenurosis in North America: Case Reports and Review. *Clinical Infectious Diseases*, *27*(3), 519–523. https://doi.org/10.1086/514716

James, E., & Boyd, W. (1937). Echinococcus Alveolaris: (With the Report of a Case). *Canadian Medical Association Journal*, *36*(4), 354–356.

James, H. A. (1968). Studies on the genus Mesocestoides (Cestoda:Cyclophyllidea) [Ph.D., Iowa State University]. In *ProQuest Dissertations and Theses* (302296927). ProQuest One Academic. https://er.lib.k-state.edu/login?url=https://www.proquest.com/dissertations-theses/studies-on-genus-mesocestoides-cestoda/docview/302296927/se-2?accountid=11789

Jenkins, D. J., Gasser, R. B., Zeyhle, E., Romig, T., & Macpherson, C. N. L. (1990). Assessment of a serological test for the detection of Echinococcus granulosus infection in dogs in Kenya. *Acta Tropica*, *47*(4), 245–248. https://doi.org/10.1016/0001-706X(90)90016-S

Jenkins, E. J., Kolapo, T. U., Jarque, M. P., Ruschkowski, C., & Frey, C. (2023). Intestinal infection with Echinococcus multilocularis in a dog. *Journal of the American Veterinary Medical Association*, *261*(9), 1–3. https://doi.org/10.2460/javma.23.02.0099

Jenkins, E. J., Peregrine, A. S., Hill, J. E., Somers, C., Gesy, K., Barnes, B., Gottstein, B., & Polley, L. (2012). Detection of European Strain of *Echinococcus multilocularis* in North America. *Emerging Infectious Diseases*, *18*(6). https://doi.org/10.3201/eid1806.111420

Jeon, H.-K., Kim, K.-H., & Eom, K. S. (2007). Complete sequence of the mitochondrial genome of Taenia saginata: Comparison with T. solium and T. asiatica. *Parasitology International*, *56*(3), 243–246. https://doi.org/10.1016/j.parint.2007.04.001

Jesudoss Chelladurai, J., Kifleyohannes, T., Scott, J., & Brewer, M. T. (2018). Praziquantel Resistance in the Zoonotic Cestode Dipylidium caninum. *The American Journal of Tropical Medicine and Hygiene*, *99*(5), 1201–1205. https://doi.org/10.4269/ajtmh.18-0533

Jesudoss Chelladurai, J. R. J., Abraham, A., Quintana, T. A., Ritchie, D., & Smith, V. (2023). Comparative Genomic Analysis and Species Delimitation: A Case for Two Species in the Zoonotic Cestode Dipylidium caninum. *Pathogens*, *12*(5), 675. https://doi.org/10.3390/pathogens12050675

Jesudoss Chelladurai, J. R. J., & Brewer, M. T. (2021). Global prevalence of Mesocestoides infections in animals – A systematic review and meta-analysis. *Veterinary Parasitology*, *298*, 109537. https://doi.org/10.1016/j.vetpar.2021.109537

Jimenez Castro, P. D., Durrence, K., Durrence, S., Gianechini, L. S., Collins, J., Dunn, K., & Kaplan, R. M. (2023). Multiple anthelmintic drug resistance in hookworms (Ancylostoma caninum) in a Labrador breeding and training kennel in Georgia, USA. *Journal of the American Veterinary Medical Association*, *261*(3), 342–347. https://doi.org/10.2460/javma.22.08.0377

Johnston, J. H., & Twente, G. E. (1952). Pulmonary hydatid (echinococcic) cyst; report of native case. *Annals of Surgery*, *136*(2), 305–308. https://doi.org/10.1097/00000658-195208000-00016

Jones, A., & Pybus, M. J. (2000). Taeniasis and Echinococcosis. In W. M. Samuel, M. J. Pybus, & A. A. Kocan (Eds.), *Parasitic Diseases of Wild Mammals* (2nd ed., p. 570). John Wiley & Sons, Inc.

Jull, P., Browne, E., Boufana, B. S., Schöniger, S., & Davies, E. (2012). Cerebral coenurosis in a cat caused by *Taenia serialis*: Neurological, magnetic resonance imaging and pathological features. *Journal of Feline Medicine and Surgery*, *14*(9), 646–649. https://doi.org/10.1177/1098612X12458211

Kaisar, M. M. M., Brienen, E. A. T., Djuardi, Y., Sartono, E., Yazdanbakhsh, M., Verweij, J. J., Supali, T., & Van Lieshout, L. (2017). Improved diagnosis of *Trichuris trichiura* by using a bead-beating procedure on ethanol preserved stool samples prior to DNA isolation and the performance of multiplex real-time PCR for intestinal parasites. *Parasitology*, *144*(7), 965–974. https://doi.org/10.1017/S0031182017000129

Karamon, J., Stojecki, K., Samorek-Pierog, M., Bilska-Zajac, E., Rozycki, M., Chmurzynska, E., Sroka, J., Zdybel, J., & Cencek, T. (2017). Genetic diversity of Echinococcus multilocularis in red foxes in Poland: The first report of a haplotype of probable Asian origin. *Folia Parasitologica*, *64*. https://doi.org/10.14411/fp.2017.007

Katz, A. M. (1958). Echinococcus disease in the United States. *The American Journal of Medicine*, *25*(5), 759–770.

Katz, R., Murphy, S., & Kosloske, A. (1980). Pulmonary echinococcosis: A pediatric disease of the Southwestern United States. *Pediatrics*, *65*(5), 1003–1006.

Kentucky Fish & Wildlife. (2024, July 17). *Reporting the first detection of E. multilocularis in Kentucky* [Government]. Kentucky Fish and Wildlife. https://fw.ky.gov/Wildlife/Pages/Echinococcus-in-Kentucky.aspx#:~:text=MULTILOCULARIS%20IN%20KENTUCKY&text=%E2%80%8BThe%20Kentucky%20Department%20of,multilocularis%20in%20Kentucky.

Kern, P. (2004). [Questions about Echinococcus multilocularis. Transmission by fallen fruit?]. *MMW Fortschritte der Medizin*, *146*(7), 18.

Kikuchi, T., & Maruyama, H. (2020). Human proliferative sparganosis update. *Parasitology International*, *75*, 102036. https://doi.org/10.1016/j.parint.2019.102036

Klein, C., Liccioli, S., & Massolo, A. (2014). Egg intensity and freeze-thawing of fecal samples affect sensitivity of Echinococcus multilocularis detection by PCR. *Parasitology Research*, *113*(10), 3867–3873. https://doi.org/10.1007/s00436-014-4055-x

Knapp, J., Bart, J. M., Glowatzki, M. L., Ito, A., Gerard, S., Maillard, S., Piarroux, R., & Gottstein, B. (2007). Assessment of Use of Microsatellite Polymorphism Analysis for Improving Spatial Distribution Tracking of *Echinococcus multilocularis*. *Journal of Clinical Microbiology*, *45*(9), 2943–2950. https://doi.org/10.1128/JCM.02107-06

Kolapo, T. U. (2023). *Molecular epidemiology and diagnostic for Echinococcus multilocularis in canid definitive and intermediate hosts* [Thesis]. University of Saskatchewan.

Kolapo, T. U., Bouchard, É., Wu, J., Bassil, M., Revell, S., Wagner, B., Acker, J. P., & Jenkins, E. J. (2021a). Copro-polymerase chain reaction has higher sensitivity compared to centrifugal fecal flotation in the diagnosis of taeniid cestodes, especially Echinococcus spp, in canids. *Veterinary Parasitology*, *292*, 109400. https://doi.org/10.1016/j.vetpar.2021.109400

Kolapo, T. U., Bouchard, É., Wu, J., Bassil, M., Revell, S., Wagner, B., Acker, J. P., & Jenkins, E. J. (2021b). Copro-polymerase chain reaction has higher sensitivity compared to centrifugal fecal flotation in the diagnosis of taeniid cestodes, especially Echinococcus spp, in canids. *Veterinary Parasitology*, *292*, 109400. https://doi.org/10.1016/j.vetpar.2021.109400

Komisarof, J. A., Olthoff, K., Siegelman, E. S., Lawton, T. J., & Furth, E. E. (2000). Focal nodular hyperplasia contiguous with an echinococcal cyst. *The American Journal of Gastroenterology*, *95*(4), 1078–1081. https://doi.org/10.1111/j.1572-0241.2000.01947.x

Konyaev, S. V., Yanagida, T., Ingovatova, G. M., Shoikhet, Y. N., Nakao, M., Sako, Y., Bondarev, A. Y., & Ito, A. (2012). Molecular identification of human echinococcosis in the Altai region of Russia. *Parasitology International*, *61*(4), 711–714. https://doi.org/10.1016/j.parint.2012.05.009

Kornfeld, H., & Mark, E. J. (1999). Case 29-1999: A 34-Year-Old Woman with One Cystic Lesion in Each Lung. *New England Journal of Medicine*, *341*(13), 974–982. https://doi.org/10.1056/NEJM199909233411308

Kotwa, J. D., Isaksson, M., Jardine, C. M., Campbell, G. D., Berke, O., Pearl, D. L., Mercer, N. J., Osterman-Lind, E., & Peregrine, A. S. (2019). *Echinococcus multilocularis* Infection, Southern Ontario, Canada. *Emerging Infectious Diseases*, *25*(2), 265–272. https://doi.org/10.3201/eid2502.180299

Kritsky, D. C., & Leiby, P. D. (1978). Studies on Sylvatic Echinococcosis. V. Factors Influencing Prevalence of Echinococcus multilocularis Leuckart 1863, in Red Foxes from North Dakota, 1965-1972. *The Journal of Parasitology*, *64*(4), 625. https://doi.org/10.2307/3279949

Kuchta, R., Kołodziej-Sobocińska, M., Brabec, J., Młocicki, D., Sałamatin, R., & Scholz, T. (2021). Sparganosis (*Spirometra*) in Europe in the Molecular Era. *Clinical Infectious Diseases*, *72*(5), 882–890. https://doi.org/10.1093/cid/ciaa1036

Kuchta, R., Phillips, A. J., & Scholz, T. (2024). Diversity and biology of Spirometra tapeworms (Cestoda: Diphyllobothriidea), zoonotic parasites of wildlife: A review. *International Journal for Parasitology: Parasites and Wildlife*, *24*, 100947. https://doi.org/10.1016/j.ijppaw.2024.100947

Kuchta, R., & Scholz, T. (2017). Diphyllobothriidea. In J. N. Caira & K. Jensen (Eds.), *Planetary Biodiversity Inventory (2008-2017): Tapeworms from Vertebrate Bowels of the Earth* (Special publication no. 25, pp. 167–189). Natural History Museum, the University of Kansas.

Kuchta, R., Scholz, T., Brabec, J., & Bray, R. A. (2008). Suppression of the tapeworm order Pseudophyllidea (Platyhelminthes: Eucestoda) and the proposal of two new orders, Bothriocephalidea and Diphyllobothriidea. *International Journal for Parasitology*, *38*(1), 49–55. https://doi.org/10.1016/j.ijpara.2007.08.005

Kuchta, R., Scholz, T., Brabec, J., & Narduzzi-Wicht, B. (2015). Diphyllobothrium, diplogonoporus, and spirometra. In L. Xiao, U. Ryan, & Y. Feng (Eds.), *Biology of Foodborne Parasites* (0 ed., pp. 299–326). CRC Press. https://doi.org/10.1201/b18317

Kuroki, K., Morishima, Y., Dorr, L., & Cook, C. R. (2022). Alveolar echinococcosis in a dog in Missouri, USA. *Journal of Veterinary Diagnostic Investigation*, *34*(4), 746–751. https://doi.org/10.1177/10406387221104754

Kuroki, K., Morishima, Y., Neil, J., Beerntsen, B. T., Matsumoto, J., & Stich, R. W. (2020). Intestinal echinococcosis in a dog from Missouri. *Journal of the American Veterinary Medical Association*, *256*(9), 1041–1046. https://doi.org/10.2460/javma.256.9.1041

Labuschagne, M., Beugnet, F., Rehbein, S., Guillot, J., Fourie, J., & Crafford, D. (2018). Analysis of *Dipylidium caninum* tapeworms from dogs and cats, or their respective fleas: Part 1. Molecular characterization of *Dipylidium caninum* : genetic analysis supporting two distinct species adapted to dogs and cats. *Parasite*, *25*, 30. https://doi.org/10.1051/parasite/2018028

Lahmar, S., Lahmar, S., Boufana, B., Bradshaw, H., & Craig, P. S. (2007). Screening for Echinococcus granulosus in dogs: Comparison between arecoline purgation, coproELISA and coproPCR with necropsy in pre-patent infections. *Veterinary Parasitology*, *144*(3–4), 287–292. https://doi.org/10.1016/j.vetpar.2006.10.016

Larsson, A. (2014). AliView: A fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics*, *30*(22), 3276–3278. https://doi.org/10.1093/bioinformatics/btu531

Laurimäe, T., Kronenberg, P. A., Alvarez Rojas, C. A., Ramp, T. W., Eckert, J., & Deplazes, P. (2020). Long-term (35 years) cryopreservation of *Echinococcus multilocularis* metacestodes. *Parasitology*, *147*(9), 1048–1054. https://doi.org/10.1017/S003118202000075X

Lavallée-Bourget, È.-M., Fernandez-Prada, C., Massé, A., Turgeon, P., & Arsenault, J. (2024). Prevalence and geographic distribution of Echinococcus genus in wild canids in southern Québec, Canada. *PLOS ONE*, *19*(7), e0306600. https://doi.org/10.1371/journal.pone.0306600

Lavers, G. D. (1957). Echinococcus cyst with intrabiliary rupture. *California Medicine*, *86*(4), 270–271.

Leiby, P. D., Carney, W. P., & Woods, C. E. (1970). Studies on sylvatic echinococcosis. 3. Host occurrence and geographic distribution of Echinococcus multilocularis in the north central United States. *The Journal of Parasitology*, *56*(6), 1141–1150.

Leiby, P. D., & Kritsky, D. C. (1972). Echinococcus multilocularis: A possible domestic life cycle in central North America and its public health implications. *The Journal of Parasitology*, *58*(6), 1213–1215.

Leiby, P. D., & Olsen, O. W. (1964). The Cestode Echinococcus multilocularis in Foxes in North Dakota. *Science*, *145*(3636), 1066–1066. https://doi.org/10.1126/science.145.3636.1066

Leutenegger, C. M., Lozoya, C. E., Tereski, J., Savard, C., Ogeer, J., & Lallier, R. (2023). Emergence of Ancylostoma caninum parasites with the benzimidazole resistance F167Y polymorphism in the US dog population. *International Journal for Parasitology: Drugs and Drug Resistance*, *21*, 131–140. https://doi.org/10.1016/j.ijpddr.2023.01.001

Lev-Tzion, R., & Goldbart, A. D. (2012). Endobronchial echinococcosis presenting as non-resolving pneumonia. *Pediatric Pulmonology*, *47*(7), 716–718. https://doi.org/10.1002/ppul.21597

Lightowlers, M. W., Gasser, R. B., Hemphill, A., Romig, T., Tamarozzi, F., Deplazes, P., Torgerson, P. R., Garcia, H. H., & Kern, P. (2021). Advances in the treatment, diagnosis, control and scientific understanding of taeniid cestode parasite infections over the past 50 years. *International Journal for Parasitology*, *51*(13–14), 1167–1192. https://doi.org/10.1016/j.ijpara.2021.10.003

Lillis, W. G., & Burrows, R. B. (1964). Natural Infections of Spirometra mansonoides in New Jersey cats. *The Journal of Parasitology*, *50*, 680.

Lin, S., Dixon, T. C., Khan, H. H., Munden, M. M., & Anderson, J. N. (2024). Unique sequelae of portal vein thrombosis in a pediatric patient with cystic echinococcosis: A case report. *JPGN Reports*, *5*(2), 218–222. https://doi.org/10.1002/jpr3.12066

Little, S., Adolph, C., Downie, K., Snider, T., & Reichard, M. (2015). High Prevalence of Covert Infection With Gastrointestinal Helminths in Cats. *Journal of the American Animal Hospital Association*, *51*(6), 359–364. https://doi.org/10.5326/JAAHA-MS-6221

Little, S., Braff, J., Duncan, K., Elsemore, D., Hanna, R., Hanscom, J., Lee, A., Martin, K. A., Sobotyk, C., Starkey, L., Sundstrom, K., Tyrrell, P., Verocai, G. G., Wu, T., & Beall, M. (2023). Diagnosis of canine intestinal parasites: Improved detection of Dipylidium caninum infection through coproantigen testing. *Veterinary Parasitology*, *324*, 110073. https://doi.org/10.1016/j.vetpar.2023.110073

Liu, C., Zhang, H., Yin, J., & Hu, W. (2015). In vivo and in vitro efficacies of mebendazole, mefloquine and nitazoxanide against cyst echinococcosis. *Parasitology Research*, *114*(6), 2213–2222. https://doi.org/10.1007/s00436-015-4412-4

Liu, I. K., Schwabe, C. W., Schantz, P. M., & Allison, M. N. (1970). The occurrence of Echinococcus granulosus in coyotes (Canis latrans) in the central valley of California. *The Journal of Parasitology*, *56*(6), 1135–1137.

Liu, Q., Li, M.-W., Wang, Z.-D., Zhao, G.-H., & Zhu, X.-Q. (2015). Human sparganosis, a neglected food borne zoonosis. *The Lancet Infectious Diseases*, *15*(10), 1226–1235. https://doi.org/10.1016/S1473-3099(15)00133-4

Lloyd, J. B., Koep, L. J., Yu, E., & Jensen, L. A. (2014). Hepatic cystic echinococcosis. *The Journal of the American Osteopathic Association*, *114*(6), 505. https://doi.org/10.7556/jaoa.2014.069

Loftus, J. P., Acevedo, A., Bowman, D. D., Liotta, J. L., Wu, T., & Zhu, M. (2022). Elimination of probable praziquantel-resistant Dipylidium caninum with nitroscanate in a mixed-breed dog: A case report. *Parasites & Vectors*, *15*(1), 438. https://doi.org/10.1186/s13071-022-05559-2

Loos-Frank, B. (1990). Cestodes of the genus Mesocestoides (Mesocestoididae) from carnivores in Isreal. *Israel Journal of Zoology*, *37*(1), 3–13. https://doi.org/10.1080/00212210.1990.10688637

Loos-Frank, B. (2000). An up-date of Verster's (1969) `Taxonomic revision of the genus Taenia Linnaeus' (Cestoda) in table format. *Systematic Parasitology*, *45*(3), 155–184. https://doi.org/10.1023/A:1006219625792

Loveless, R. M., Andersen, F. L., Ramsay, M. J., & Hedelius, R. K. (1978). Echinococcus granulosus in dogs and sheep in central Utah, 1971-1976. *American Journal of Veterinary Research*, *39*(3), 499–502.

Low, V. L., Prakash, B. K., Tan, T. K., Sofian-Azirun, M., Anwar, F. H. K., Vinnie-Siow, W. Y., & AbuBakar, S. (2017). Pathogens in ectoparasites from free-ranging animals: Infection with Rickettsia asembonensis in ticks, and a potentially new species of Dipylidium in fleas and lice. *Veterinary Parasitology*, *245*, 102–105. https://doi.org/10.1016/j.vetpar.2017.08.015

Mahanty, S., & Garcia, H. H. (2010). Cysticercosis and neurocysticercosis as pathogens affecting the nervous system. *Progress in Neurobiology*, *91*(2), 172–184. https://doi.org/10.1016/j.pneurobio.2009.12.008

Massolo, A., Klein, C., Kowalewska-Grochowska, K., Belga, S., MacDonald, C., Vaughan, S., Girgis, S., Giunchi, D., Bramer, S. A., Santa, M. A., Grant, D. M., Mori, K., Duignan, P., Slater, O., Gottstein, B., Müller, N., & Houston, S. (2019). European *Echinococcus multilocularis* Identified in Patients in Canada. *New England Journal of Medicine*, *381*(4), 384–385. https://doi.org/10.1056/NEJMc1814975

Massolo, A., Liccioli, S., Budke, C., & Klein, C. (2014). *Echinococcus multilocularis* in North America: The great unknown. *Parasite*, *21*, 73. https://doi.org/10.1051/parasite/2014069

Mathis, A., Deplazes, P., & Eckert, J. (1996). An Improved test system for PCR-based specific detection of EM eggs. *Journal of Helminthology*, *70*, 219–222. https://doi.org/10.1017/S0022149X00015443

McAllister, C. T., & Conn, D. B. (1990). Occurrence of Tetrathyridia of Mesocestoides sp. (Cestoidea: Cyclophyllidea) in North American Anurans (Amphibia). *Journal of Wildlife Diseases*, *26*(4), 540–543. https://doi.org/10.7589/0090-3558-26.4.540

McAllister, C. T., Tkach, V. V., & Conn, D. B. (2018). Morphological and Molecular Characterization of Post-Larval Pre-Tetrathyridia of *Mesocestoides* sp. (Cestoda: Cyclophyllidea) from Ground Skink, *Scincella lateralis* (SAURIA: SCINCIDAE), FROM SOUTHEASTERN OKLAHOMA. *Journal of Parasitology*, *104*(3), 246–253. https://doi.org/10.1645/17-178

McGarry, J., Collins, M., & Baross, K. (2020). UK report of tapeworm *Mesocestoides litteratus*. *Veterinary Record*, *186*(15), 498–499. https://doi.org/10.1136/vr.m1689

McHale, B., Callahan, R. T., Paras, K. L., Weber, M., Kimbrell, L., Velázquez-Jiménez, Y., McManamon, R., Howerth, E. W., & Verocai, G. G. (2020). Sparganosis due to Spirometra sp. (Cestoda; Diphyllobothriidae) in captive meerkats (Suricata suricatta). *International Journal for Parasitology: Parasites and Wildlife*, *13*, 186–190. https://doi.org/10.1016/j.ijppaw.2020.10.005

Melotti, J. R. (2013). *A survey for Echinococcus multilocularis in coyotes and foxes in Michigan* [Thesis, Michigan State University]. https://er.lib.k-state.edu/login?url=https://www.proquest.com/dissertations-theses/survey-i-echinococcus-multilocularis-coyotes/docview/1476202982/se-2

Melotti, J. R., Muzzall, P. M., O'Brien, D. J., Cooley, T. M., & Tsao, J. I. (2015). Low Prevalence of *Echinococcus multilocularis* in Michigan, U.S.A.: A Survey of Coyotes (*Canis latrans*), Red Foxes (*Vulpes vulpes*), and Gray Foxes (*Urocyon cinereoargenteus*), 2009–2012. *Comparative Parasitology*, *82*(2), 285–290. https://doi.org/10.1654/4752.1

Miller, H. E., & Collins, C. G. (1937). ECHINOCOCCUS DISEASE: REPORT OF A CASE OF PRIMARY ECHINOCOCCUS CYST OF THE UTERUS. *Annals of Surgery*, *105*(6), 886–895. https://doi.org/10.1097/00000658-193706000-00002

Molina, C. P., Ogburn, J., & Adegboyega, P. (2003). Infection by *Dipylidium caninum* in an Infant. *Archives of Pathology & Laboratory Medicine*, *127*(3), e157–e159. https://doi.org/10.5858/2003-127-e157-IBDCIA

Montalbano Di Filippo, M., Meoli, R., Cavallero, S., Eleni, C., De Liberato, C., & Berrilli, F. (2018). Molecular identification of Mesocestoides sp. Metacestodes in a captive goldhanded tamarin (Saguinus midas). *Infection, Genetics and Evolution*, *65*, 399–405. https://doi.org/10.1016/j.meegid.2018.08.008

Monteiro, L., Bonnemaison, D., Vekris, A., Petry, K. G., Bonnet, J., Vidal, R., Cabrita, J., & Mégraud, F. (1997). Complex polysaccharides as PCR inhibitors in feces: Helicobacter pylori model. *Journal of Clinical Microbiology*, *35*(4), 995–998. https://doi.org/10.1128/jcm.35.4.995-998.1997

Muchaamba, G., Alvarez Rojas, C. A., & Deplazes, P. (2021). Amplification of cestode DNA from the peri-anal region of naturally infected foxes by PCR and LAMP: Proof of concept for a potential sampling strategy for diagnosing human taeniosis. *Parasitology Research*, *120*(10), 3451–3459. https://doi.org/10.1007/s00436-021-07271-z

Mueller, J. F. (1974). The biology of Spirometra. *The Journal of Parasitology*, *60*(1), 3–14.

Mueller, J. F., Hart, E. P., & Walsh, W. P. (1963). Human Sparganosis in the United States. *The Journal of Parasitology*, *49*(2), 294. https://doi.org/10.2307/3275998

Muller, R., Muller, R., & Wakelin, D. (2002). *Worms and human disease* (2nd ed). CABI.

Murali, M. R., Uyeda, J. W., & Tingpej, B. (2015). Case 2-2015: A 25-Year-Old Man with Abdominal Pain, Syncope, and Hypotension. *New England Journal of Medicine*, *372*(3), 265–273. https://doi.org/10.1056/NEJMcpc1410939

Nagamori, Y., Payton, M. E., Looper, E., Apple, H., & Johnson, E. M. (2020a). Retrospective survey of endoparasitism identified in feces of client-owned dogs in North America from 2007 through 2018. *Veterinary Parasitology*, *282*, 109137. https://doi.org/10.1016/j.vetpar.2020.109137

Nagamori, Y., Payton, M. E., Looper, E., Apple, H., & Johnson, E. M. (2020b). Retrospective survey of parasitism identified in feces of client-owned cats in North America from 2007 through 2018. *Veterinary Parasitology*, *277*, 109008. https://doi.org/10.1016/j.vetpar.2019.109008

Nakao, M., Xiao, N., Okamoto, M., Yanagida, T., Sako, Y., & Ito, A. (2009a). Geographic pattern of genetic variation in the fox tapeworm Echinococcus multilocularis. *Parasitology International*, *58*(4), 384–389. https://doi.org/10.1016/j.parint.2009.07.010

Nakao, M., Xiao, N., Okamoto, M., Yanagida, T., Sako, Y., & Ito, A. (2009b). Geographic pattern of genetic variation in the fox tapeworm Echinococcus multilocularis. *Parasitology International*, *58*(4), 384–389. https://doi.org/10.1016/j.parint.2009.07.010

Narain, J. P., Bradsher, R., Sanders, C. R., & Lofgren, J. P. (1986). Unilocular hydatid disease in Arkansas. *Southern Medical Journal*, *79*(6), 781–782. https://doi.org/10.1097/00007611-198606000-00038

Nash, T. E., Singh, G., White, A. C., Rajshekhar, V., Loeb, J. A., Proaño, J. V., Takayanagui, O. M., Gonzalez, A. E., Butman, J. A., DeGiorgio, C., Del Brutto, O. H., Delgado-Escueta, A., Evans, C. A. W., Gilman, R. H., Martinez, S. M., Medina, M. T., Pretell, E. J., Teale, J., & Garcia, H. H. (2006). Treatment of neurocysticercosis: Current status and future research needs. *Neurology*, *67*(7), 1120–1127. https://doi.org/10.1212/01.wnl.0000238514.51747.3a

Nguyen, L.-T., Schmidt, H. A., Von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Molecular Biology and Evolution*, *32*(1), 268–274. https://doi.org/10.1093/molbev/msu300

Ntoukas, V., Tappe, D., Pfütze, D., Simon, M., & Holzmann, T. (2013). Cerebellar Cysticercosis Caused by Larval *Taenia crassiceps* Tapeworm in Immunocompetent Woman, Germany. *Emerging Infectious Diseases*, *19*(12), 2008–2011. https://doi.org/10.3201/eid1912.130284

Nunnari, G. (2012). Hepatic echinococcosis: Clinical and therapeutic aspects. *World Journal of Gastroenterology*, *18*(13), 1448. https://doi.org/10.3748/wjg.v18.i13.1448

Oehm, A. W., Reiter, A., Binz, A., & Schnyder, M. (2024). First report of apparent praziquantel resistance in *Dipylidium caninum* in Europe. *Parasitology*, *151*(5), 523–528. https://doi.org/10.1017/S0031182024000398

Padgett, K. A. (1991). *Life Cycles and Systematics of Mesocestoides spp. Tapeworms* [Ph.D.]. University of California Davis.

Padgett, K. A., & Boyce, W. M. (2005). Ants as first intermediate hosts of *Mesocestoides* on San Miguel Island, USA. *Journal of Helminthology*, *79*(1), 67–73. https://doi.org/10.1079/JOH2005275

Padgett, K. A., Nadler, S. A., Munson, L., Sacks, B., & Boyce, W. M. (2005). SYSTEMATICS OF MESOCESTOIDES (CESTODA: MESOCESTOIDIDAE): EVALUATION OF MOLECULAR AND MORPHOLOGICAL VARIATION AMONG ISOLATES. *Journal of Parasitology*, *91*(6), 1435–1443. https://doi.org/10.1645/GE-3461.1

Papini, R., Matteini, A., Bandinelli, P., Pampurini, F., & Mancianti, F. (2010). Effectiveness of praziquantel for treatment of peritoneal larval cestodiasis in dogs: A case report. *Veterinary Parasitology*, *170*(1–2), 158–161. https://doi.org/10.1016/j.vetpar.2010.02.001

Park, S.-K., & Marchant, J. S. (2020). The Journey to Discovering a Flatworm Target of Praziquantel: A Long TRP. *Trends in Parasitology*, *36*(2), 182–194. https://doi.org/10.1016/j.pt.2019.11.002

Parwani, A. V., Burroughs, F. H., & Ali, S. Z. (2004). Echinococcal cyst of the liver. *Diagnostic Cytopathology*, *31*(2), 111–112. https://doi.org/10.1002/dc.20026

Passarelli, P., Ramchandar, N., Naheedy, J., Kling, K., Choi, L., & Pong, A. (2022). AN 8-YEAR-OLD CALIFORNIA GIRL WITH ASYMPTOMATIC HEPATIC CYSTS.

Pediatric Infectious Disease Journal, *41*(7), e295–e296. https://doi.org/10.1097/INF.0000000000003539

Peregrine, A. S., Jenkins, E. J., Barnes, B., Johnson, S., Polley, L., Barker, I. K., Wolf, B. D., & Gottstein, B. (2012). *Case Report   Rapport de cas*. *53*, 5.

Piarroux, M., Piarroux, R., Knapp, J., Bardonnet, K., Dumortier, J., Watelet, J., Gerard, A., Beytout, J., Abergel, A., Bresson-Hadni, S., Gaudart, J., & for the FrancEchino Surveillance Network. (2013). Populations at Risk for Alveolar Echinococcosis, France. *Emerging Infectious Diseases*, *19*(5), 721–728. https://doi.org/10.3201/eid1905.120867

Pinch, L. W., & Wilson, J. F. (1973). Non-Surgical Management of Cystic Hydatid Disease in Alaska: A Review of 30 Cases of Echinococcus Granulosus Infection Treated without Operation. *Annals of Surgery*, *178*(1), 45–48. https://doi.org/10.1097/00000658-197307000-00010

Pipas, M. J., Fowler, D. R., Bardsley, K. D., & Bangoura, B. (2021). Survey of coyotes, red foxes and wolves from Wyoming, USA, for Echinococcus granulosus s. L. *Parasitology Research*, *120*(4), 1335–1340. https://doi.org/10.1007/s00436-021-07059-1

Polish, L. B., O'Connell, E. M., Barth, T. F. E., Gottstein, B., Zajac, A., Gibson, P. C., Bah, A., Kirchgessner, M., Estrada, M., Seguin, M. A., & Ramirez-Barrios, R. (2022). European Haplotype of *Echinococcus multilocularis* in the United States. *New England Journal of Medicine*, *387*(20), 1902–1904. https://doi.org/10.1056/NEJMc2210000

Polish, L. B., Pritt, B., Barth, T. F. E., Gottstein, B., O'Connell, E. M., & Gibson, P. C. (2021). First European Haplotype of *Echinococcus multilocularis* Identified in the United States: An Emerging Disease? *Clinical Infectious Diseases*, *72*(7), 1117–1123. https://doi.org/10.1093/cid/ciaa245

Priest, J. M., McRuer, D. L., Stewart, D. T., Boudreau, M., Power, J. W. B., Conboy, G., Jenkins, E. J., Kolapo, T. U., & Shutler, D. (2021). New geographic records for Echinococcus canadensis in coyotes and moose from Nova Scotia, Canada. *International Journal for Parasitology: Parasites and Wildlife*, *16*, 285–288. https://doi.org/10.1016/j.ijppaw.2021.11.004

Rausch, R. L. (1960). *Recent Studies on Hydatid Disease in Alaska. 2*(3), 391–398.

Rausch, R. L., & Richards, S. H. (1971). Observations on parasite–host relationships of *Echinococcus multilocularis* Leuckart, 1863, in North Dakota. *Canadian Journal of Zoology*, *49*(10), 1317–1330. https://doi.org/10.1139/z71-198

Rausch, R., & Schiller, E. L. (1951). Hydatid Disease (*Echinococcosis*) in Alaska and the Importance of Rodent Intermediate Hosts. *Science*, *113*(2925), 57–58. https://doi.org/10.1126/science.113.2925.57

Rausch, R., & Williamson, F. S. (1959). Studies on the helminth fauna of Alaska. XXXIV. The parasites of wolves, Canis lupus L. *The Journal of Parasitology*, *45*(4, Part 1), 395–403.

Rebusi, N. (n.d.). *Peri-Operative Diagnosis of Isolated CNS Echinococcus presenting as Seizure and Headache* [Poster]. Retrieved April 11, 2025, from https://www.acponline.org/sites/default/files/documents/about_acp/chapters/me/rebusi_nicole_pgy_2.pdf

Redman, W. K., Bryant, J. E., & Ahmad, G. (2016). Gastrointestinal helminths of Coyotes (Canis latrans) from Southeast Nebraska and Shenandoah area of Iowa. *Veterinary World*, *9*(9), 970–975. https://doi.org/10.14202/vetworld.2016.970-975

Richards, R., & Somerville, J. (1980). Field trials with nitroscanate against cestodes and nematodes in dogs. *Veterinary Record*, *106*(15), 332–335. https://doi.org/10.1136/vr.106.15.332

Riviere, J. E., & Papich, M. G. (2018). *Veterinary Pharmacology and Therapeutics*. John Wiley & Sons, Incorporated. http://ebookcentral.proquest.com/lib/ksu/detail.action?docID=5167239

Romig, T., Deplazes, P., Jenkins, D., Giraudoux, P., Massolo, A., Craig, P. S., Wassermann, M., Takahashi, K., & de la Rue, M. (2017). Ecology and Life Cycle Patterns of Echinococcus Species. In *Advances in Parasitology* (Vol. 95, pp. 213–314). Elsevier. https://doi.org/10.1016/bs.apar.2016.11.002

Rousseau, J., Castro, A., Novo, T., & Maia, C. (2022). Dipylidium caninum in the twenty-first century: Epidemiological studies and reported cases in companion animals and humans. *Parasites & Vectors*, *15*(1), 131. https://doi.org/10.1186/s13071-022-05243-5

Rust, M. (2017). The Biology and Ecology of Cat Fleas and Advancements in Their Pest Management: A Review. *Insects*, *8*(4), 118. https://doi.org/10.3390/insects8040118

Rybolt, L., Oehler, R. L., Khalil, F. K., Khalil, B., & Toney, J. F. (2024). A 22-Year-Old With a Left Apical Chest Mass. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *78*(1), 199–201. https://doi.org/10.1093/cid/ciad524

Saarma, U., Skirnisson, K., Björnsdottir, T. S., Laurimäe, T., & Kinkar, L. (2023). Cystic echinococcosis in Iceland: A brief history and genetic analysis of a 46-year-old *Echinococcus* isolate collected prior to the eradication of this zoonotic disease. *Parasitology*, *150*(7), 638–643. https://doi.org/10.1017/S0031182023000355

Saini, V. K., Gupta, S., Kasondra, A., Rakesh, R. L., & Latchumikanthan, A. (2016). Diagnosis and therapeutic management of Dipylidium caninum in dogs: A case report. *Journal of Parasitic Diseases*, *40*(4), 1426–1428. https://doi.org/10.1007/s12639-015-0706-9

Samuel, W. M., Ramalingam, S., & Carbyn, L. N. (1978). Helminths in coyotes (*Canis latrans* Say), wolves (*Canis lupus* L.), and red foxes (*Vulpes vulpes* L.) of southwestern Manitoba. *Canadian Journal of Zoology*, *56*(12), 2614–2617. https://doi.org/10.1139/z78-351

Sawitz, W. (1938). Echinococcus Infection in Louisiana. *The Journal of Parasitology*, *24*(5), 437. https://doi.org/10.2307/3272120

Sawyer, J. C., Schantz, P. M., Schwabe, C. W., & Newbold, M. W. (1969). Identification of transmission foci of hydatid disease in California. *Public Health Reports (Washington, D.C.: 1896)*, *84*(6), 531–541.

Schantz, P. M., Clérou, R. P., Liu, I. K. M., & Schwabe, C. W. (1970). Hydatid Disease in the Central Valley of California: Transmission of Infection among Dogs, Sheep, and Man in Kern County. *The American Journal of Tropical Medicine and Hygiene*, *19*(5), 823–830. https://doi.org/10.4269/ajtmh.1970.19.823

Schantz, P. M., Van Alstine, C., Blacksheep, A., & Sinclair, S. (1977). Prevalence of Echinococcus granulosus and Other Cestodes in Dogs on the Navajo Reservation in Arizona and New Mexico. *American Journal of Veterinary Research*, *38*(5), 669–670. https://doi.org/10.2460/ajvr.1977.38.05.669

Schantz, P. M., von Reyn, C. F., Welty, T., Andersen, F. L., Schultz, M. G., & Kagan, I. G. (1977). Epidemiologic investigation of echinococcosis in American Indians living in Arizona and New Mexico. *The American Journal of Tropical Medicine and Hygiene*, *26*(1), 121–126. https://doi.org/10.4269/ajtmh.1977.26.121

Schantz, P. M., Von Reyn, C. F., Welty, T., & Schultz, M. G. (1976). Echinococcosis in Arizona and New Mexico: Survey of Hospital Records, 1969–1974. *The American Journal of Tropical Medicine and Hygiene*, *25*(2), 312–317. https://doi.org/10.4269/ajtmh.1976.25.312

Schmidt, G. D. (1986). *CRC Handbook of tapeworm identification.* CRC Press, Inc.

Scholz, T., & Kuchta, R. (2016). Fish-borne, zoonotic cestodes (Diphyllobothrium and relatives) in cold climates: A never-ending story of neglected and (re)-emergent parasites. *Food and Waterborne Parasitology*, *4*, 23–38. https://doi.org/10.1016/j.fawpar.2016.07.002

Scholz, T., Kuchta, R., & Brabec, J. (2019). Broad tapeworms (Diphyllobothriidae), parasites of wildlife and humans: Recent progress and future challenges. *International Journal for Parasitology: Parasites and Wildlife*, *9*, 359–369. https://doi.org/10.1016/j.ijppaw.2019.02.001

Schurer, J. M., Bouchard, E., Bryant, A., Revell, S., Chavis, G., Lichtenwalner, A., & Jenkins, E. J. (2018). Echinococcus in wild canids in Québec (Canada) and Maine (USA). *PLOS Neglected Tropical Diseases*, *12*(8), e0006712. https://doi.org/10.1371/journal.pntd.0006712

Schurer, J., Shury, T., Leighton, F., & Jenkins, E. (2013). Surveillance for Echinococcus canadensis genotypes in Canadian ungulates. *International Journal for Parasitology: Parasites and Wildlife*, *2*, 97–101. https://doi.org/10.1016/j.ijppaw.2013.02.004

Schweiger, A., Ammann, R. W., Candinas, D., Clavien, P.-A., Eckert, J., Gottstein, B., Halkic, N., Muellhaupt, B., Prinz, B. M., Reichen, J., Tarr, P. E., Torgerson, P. R., & Deplazes, P. (2007). Human Alveolar Echinococcosis after Fox Population Increase, Switzerland. *Emerging Infectious Diseases*, *13*(6), 878–882. https://doi.org/10.3201/eid1306.061074

Seesee, F. M., Sterner, M. C., & Worley, D. E. (1983). Helminths of the Coyote (Canis latrans Say) in Montana. *Journal of Wildlife Diseases*, *19*(1), 54–55. https://doi.org/10.7589/0090-3558-19.1.54

Shakya, A., Bhat, H. R., & Ghosh, S. K. (2018). Update on Nitazoxanide: A Multifunctional Chemotherapeutic Agent. *Current Drug Discovery Technologies*, *15*(3), 201–213. https://doi.org/10.2174/1570163814666170727130003

Siles-Lucas, M., Casulli, A., Conraths, F. J., & Müller, N. (2017). Laboratory Diagnosis of Echinococcus spp. In Human Patients and Infected Animals. In *Advances in Parasitology* (Vol. 96, pp. 159–257). Elsevier. https://doi.org/10.1016/bs.apar.2016.09.003

Siles-Lucas, M., & Hemphill, A. (2002). Cestode parasites: Application of in vivo and in vitro models for studies on the host-parasite relationship. In *Advances in Parasitology* (Vol. 51, pp. 133–230). Elsevier. https://doi.org/10.1016/S0065-308X(02)51005-8

Simpson, C., Jabbar, A., Mansfield, C. S., Tyrrell, D., Croser, E., Abraham, L. A., & Gasser, R. B. (2012). Molecular diagnosis of sparganosis associated with pneumothorax in a dog. *Molecular and Cellular Probes*, *26*(1), 60–62. https://doi.org/10.1016/j.mcp.2011.08.003

Skelding, A., Brooks, A., Stalker, M., Mercer, N., de Villa, E., Gottstein, B., & Peregrine, A. S. (2014). Hepatic alveolar hydatid disease (Echinococcus multilocularis) in a boxer dog from southern Ontario. *The Canadian Veterinary Journal = La Revue Veterinaire Canadienne*, *55*(6), 551–553.

Slocombe, R., Arundel, J., Labuc, R., & Doyle, M. (1989). Cerebral coenuriasis in a domestic cat. *Australian Veterinary Journal*, *66*(3), 92–93. https://doi.org/10.1111/j.1751-0813.1989.tb09753.x

Snyder, J. R. (1914). Echinococcus in California. *California State Journal of Medicine*, *12*(7), 294–295.

Sorvillo, F. J., DeGiorgio, C., & Waterman, S. H. (2007). Deaths from Cysticercosis, United States. *Emerging Infectious Diseases*, *13*(2), 230–235. https://doi.org/10.3201/eid1302.060527

Soulsby, E. J. L. (1968). *Helminths, arthropods and protozoa of domesticated animals.* Baillière Tindall & Cassell Ltd.

Soulsby, E. J. L. (1982). *Helminths, arthropods and protozoa of domesticated animals.* (Issue 7th edition). Bailliere Tindall, 10 Greycoat Place.

Špakulová, M., Orosová, M., & Mackiewicz, J. S. (2011). Cytogenetics and Chromosomes of Tapeworms (Platyhelminthes, Cestoda). In *Advances in Parasitology* (Vol. 74, pp. 177–230). Elsevier. https://doi.org/10.1016/B978-0-12-385897-9.00003-3

Sridhar, R., Dittmar, K., & Williams, H. M. (2022). Using Surface Washing to Remove the Environmental Component from Flea Microbiome Analysis. *Journal of Parasitology*, *108*(3). https://doi.org/10.1645/21-60

Stancampiano, L., Ravagnan, S., Capelli, G., & Militerno, G. (2019). Cysticercosis by Taenia pisiformis in Brown Hare (Lepus europaeus) in Northern Italy: Epidemiologic and pathologic features. *International Journal for Parasitology: Parasites and Wildlife*, *9*, 139–143. https://doi.org/10.1016/j.ijppaw.2019.04.004

Stiles, C. W. (1903). *A case of infection with the double-pored dog tapeworm (Dipylidium caninum) in an American child*.

Storandt, S. T., & Kazacos, K. R. (1993). Echinococcus multilocularis Identified in Indiana, Ohio, and East-central Illinois. *The Journal of Parasitology*, *79*(2), 301. https://doi.org/10.2307/3283527

Storandt, S. T., & Kazacos, K. R. (2012). Echinococcus multilocularis Identified in Michigan with Additional Records From Ohio. *Journal of Parasitology*, *98*(4), 891–893. https://doi.org/10.1645/GE-3057.1

Storandt, S. T., Virchow, D. R., Dryden, M. W., Hygnstrom, S. E., & Kazacos, K. R. (2002). Distribution and Prevalence of Echinococcus multilocularis in Wild Predators in Nebraska, Kansas, and Wyoming. *Journal of Parasitology*, *88*(2), 420–422. https://doi.org/10.1645/0022-3395(2002)088[0420:DAPOEM]2.0.CO;2

Sutherland, C. J., Tomlinson, T., Wilson, G., Gray, A., Miller, K. R., Gin, T., Lashnits, E., Li, Y., Kollasch, T. M., Locklear, C. L., Ryan, W. G., Canfield, M., & Herrin, B. H. (In Review). *Assessment of lotilaner (Credelio® CAT) for control of in-home Ctenocephalides felis infestations* [Manuscript].

Sweatman, G. K. (1952). Distribution and incidence of Echinococcus granulosus in man and other animals with special reference to Canada. *Canadian Journal of Public Health = Revue Canadienne De Sante Publique*, *43*(11), 480–486.

Taxy, J. B., Gibson, W. E., & Kaufman, M. W. (2017). Echinococcosis: Unexpected Occurrence and the Diagnostic Contribution of Routine Histopathology. *American Journal of Surgical Pathology*, *41*(1), 94–100. https://doi.org/10.1097/PAS.0000000000000742

Thomas, C. M., & Timson, D. J. (2020). The Mechanism of Action of Praziquantel: Can New Drugs Exploit Similar Mechanisms? *Current Medicinal Chemistry*, *27*(5), 676–696. https://doi.org/10.2174/0929867325666180926145537

Thomas, G., Thomas, H., Thomas, J., Brittenum, D., & Rowe, G. (1984). Hydatid disease (echinococcosis) of the lung in a community hospital: Report of a case. *The Journal of the American Osteopathic Association*, *83*(12), 859–865.

Thompson, C. A., Malcolm, J. R., & Patterson, B. R. (2021). Individual and Temporal Variation in Use of Residential Areas by Urban Coyotes. *Frontiers in Ecology and Evolution*, *9*, 687504. https://doi.org/10.3389/fevo.2021.687504

Thompson, C. W., Phelps, K. L., Allard, M. W., Cook, J. A., Dunnum, J. L., Ferguson, A. W., Gelang, M., Khan, F. A. A., Paul, D. L., Reeder, D. M., Simmons, N. B., Vanhove, M. P. M., Webala, P. W., Weksler, M., & Kilpatrick, C. W. (2021). Preserve a Voucher Specimen! The Critical Need for Integrating Natural History Collections in Infectious Disease Studies. *mBio*, *12*(1), e02698-20. https://doi.org/10.1128/mBio.02698-20

Thompson, R. C. A. (2017). Biology and Systematics of Echinococcus. In *Advances in Parasitology* (Vol. 95, pp. 65–109). Elsevier. https://doi.org/10.1016/bs.apar.2016.07.001

Torgerson, P. R., & Deplazes, P. (2009). Echinococcosis: Diagnosis and diagnostic interpretation in population studies. *Trends in Parasitology*, *25*(4), 164–170. https://doi.org/10.1016/j.pt.2008.12.008

Trachsel, D., Deplazes, P., & Mathis, A. (2007). Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA. *Parasitology*, *134*(6), 911–920. https://doi.org/10.1017/S0031182007002235

Urqhuart, G. M., Armour, J., Dunncan, J., & Jennings, F. W. (1996). *Veterinary Parasitology*.

Vande Vusse, F. J., Little, D. E., Calloway, R. B., & Ballard, N. B. (1978). Incidence and distribution of Echinococcus multilocularis in fox from southern Minnesota and Northern Iowa. *Page 93 in Program of Abstracts*. 53rd Annual Meeting of the American Association of Veterinary Parasitologists, Chicago, Illinois, USA.

Varcasia, A., Tamponi, C., Ahmed, F., Cappai, M. G., Porcu, F., Mehmood, N., Dessì, G., & Scala, A. (2022). Taenia multiceps coenurosis: A review. *Parasites & Vectors*, *15*(1), 84. https://doi.org/10.1186/s13071-022-05210-0

Venkatesan, A., Jimenez Castro, P. D., Morosetti, A., Horvath, H., Chen, R., Redman, E., Dunn, K., Collins, J. B., Fraser, J. S., Andersen, E. C., Kaplan, R. M., & Gilleard, J. S. (2023). Molecular evidence of widespread benzimidazole drug resistance in Ancylostoma caninum from domestic dogs throughout the USA and discovery of a novel β-tubulin benzimidazole resistance mutation. *PLOS Pathogens*, *19*(3), e1011146. https://doi.org/10.1371/journal.ppat.1011146

Verocai, G. G., Harvey, T. V., Sobotyk, C., Siu, R. E., Kulpa, M., & Connolly, M. (2023). Spirometra infection in a captive Samar cobra (Naja samarensis) in the United States: An imported case? *International Journal for Parasitology: Parasites and Wildlife*, *20*, 133–137. https://doi.org/10.1016/j.ijppaw.2023.02.001

Vettorazzi, R., Norbis, W., Martorelli, S. R., García, G., & Ríos, N. (2023). First report of Spirometra (Eucestoda; Diphyllobothriidae) naturally occurring in a fish host. *Folia Parasitologica*, *70*. https://doi.org/10.14411/fp.2023.008

Villeneuve, A., Polley, L., Jenkins, E., Schurer, J., Gilleard, J., Kutz, S., Conboy, G., Benoit, D., Seewald, W., & Gagné, F. (2015a). Parasite prevalence in fecal samples from shelter dogs and cats across the Canadian provinces. *Parasites & Vectors*, *8*(1), 281. https://doi.org/10.1186/s13071-015-0870-x

Villeneuve, A., Polley, L., Jenkins, E., Schurer, J., Gilleard, J., Kutz, S., Conboy, G., Benoit, D., Seewald, W., & Gagné, F. (2015b). Parasite prevalence in fecal samples from shelter dogs and cats across the Canadian provinces. *Parasites & Vectors*, *8*(1), 281. https://doi.org/10.1186/s13071-015-0870-x

Von Bonsdorff, B. (1977). *Diphyllobothriasis in man.* Academic Press Inc. (London) Ltd., 24/28 Oval Road, London NW1 7DX.

Waeschenbach, A., Brabec, J., Scholz, T., Littlewood, D. T. J., & Kuchta, R. (2017). The catholic taste of broad tapeworms – multiple routes to human infection. *International Journal for Parasitology*, *47*(13), 831–843. https://doi.org/10.1016/j.ijpara.2017.06.004

Wang, Q., Vuitton, D. A., Xiao, Y., Budke, C. M., Campos-Ponce, M., Schantz, P. M., Raoul, F., Yang, W., Craig, P. S., & Giraudoux, P. (2006). Pasture Types and *Echinococcus multilocularis* , Tibetan Communities. *Emerging Infectious Diseases*, *12*(6), 1008–1010. https://doi.org/10.3201/eid1206.041229

Wang, Q., Zhong, B., Yu, W., Zhang, G., Budke, C. M., Liao, S., He, W., Chen, F., Xu, K., Xie, F., Danbazeli, Wang, Q., Yang, L., Huang, Y., Li, R., Yao, R., Giraudoux, P., & Craig, P. S. (2021). Assessment of a 10-year dog deworming programme on the transmission of Echinococcus multilocularis in Tibetan communities in Sichuan Province, China. *International Journal for Parasitology*, *51*(2–3), 159–166. https://doi.org/10.1016/j.ijpara.2020.08.010

Wardle, R. A., McLEOD, J. A., & Stewart, I. E. (1947). Lühe's Diphyllobothrium (Cestoda). *The Journal of Parasitology*, *33*(4), 319–330.

White Jr, A. C. (2004). Nitazoxanide: A new broad spectrum antiparasitic agent. *Expert Review of Anti-Infective Therapy*, *2*(1), 43–49. https://doi.org/10.1586/14787210.2.1.43

WHO. (2021, March 17). *Echinococcosis*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/echinococcosis

Wilcox, R. S., Bowman, D. D., Barr, S. C., & Euclid, J. M. (2009). Intestinal Obstruction Caused by Taenia taeniaeformis Infection in a Cat. *Journal of the American Animal Hospital Association*, *45*(2), 93–96. https://doi.org/10.5326/0450093

Williams, L. B. A., & Walzthoni, N. (2023). Diagnosis, treatment, and outcome of four dogs with alveolar echinococcosis in the northwestern United States. *Journal of the American Veterinary Medical Association*, 1–6. https://doi.org/10.2460/javma.22.12.0540

Wilson, J. F., Diddams, A. C., & Rausch, R. L. (1968). Cystic hydatid disease in Alaska. A review of 101 autochthonous cases of Echinococcus granulosus infection. *The American Review of Respiratory Disease, 98*(1), 1–15. https://doi.org/10.1164/arrd.1968.98.1.1

Wilson, J. F., & Rausch, R. L. (1980). Alveolar hydatid disease. A review of clinical features of 33 indigenous cases of Echinococcus multilocularis infection in Alaskan Eskimos. *The American Journal of Tropical Medicine and Hygiene, 29*(6), 1340–1355.

Wirtherle, N., Wiemann, A., Ottenjann, M., Linzmann, H., Van Der Grinten, E., Kohn, B., Gruber, A. D., & Clausen, P.-H. (2007). First case of canine peritoneal larval cestodosis caused by Mesocestoides lineatus in Germany. *Parasitology International*, *56*(4), 317–320. https://doi.org/10.1016/j.parint.2007.06.006

Wobeser, G. (1971). The occurrence of Echinococcus multilocularis (Leukart, 1863) in cats near Saskatoon, Saskatchewan. *The Canadian Veterinary Journal = La Revue Veterinaire Canadienne*, *12*(3), 65–68.

Woldemeskel, M. (2014). Subcutaneous sparganosis, a zoonotic cestodiasis, in two cats. *Journal of Veterinary Diagnostic Investigation*, *26*(2), 316–319. https://doi.org/10.1177/1040638713517697

Woolsey, I. D., & Miller, A. L. (2021). Echinococcus granulosus sensu lato and Echinococcus multilocularis: A review. *Research in Veterinary Science*, *135*, 517–522. https://doi.org/10.1016/j.rvsc.2020.11.010

Wyrosdick, H. M., Chapman, A., Martinez, J., & Schaefer, J. J. (2017). Parasite prevalence survey in shelter cats in Citrus County, Florida. *Veterinary Parasitology: Regional Studies and Reports*, *10*, 20–24. https://doi.org/10.1016/j.vprsr.2017.07.002

Yamasaki, H., Nakao, M., Nakaya, K., Schantz, P. M., & Ito, A. (2008). Genetic analysis of Echinococcus multilocularis originating from a patient with alveolar echinococcosis occurring in Minnesota in 1977. *The American Journal of Tropical Medicine and Hygiene*, *79*(2), 245–247.

Yasur-Landau, D., Genad, O., Salant, H., Dvir, E., Mazuz, M. L., & Baneth, G. (2023). Comparison of multiplex copro PCR with coproscopy followed by PCR on recovered eggs for the detection of Echinococcus granulosus and Taenia spp. Infection in dogs. *Veterinary Parasitology*, *315*, 109885. https://doi.org/10.1016/j.vetpar.2023.109885

Yasur-Landau, D., Salant, H., Levin-Gichon, G., Botero-Anug, A.-M., Zafrany, A., Mazuz, M. L., & Baneth, G. (2019). Urinary incontinence associated with Mesocestoides vogae infection in a dog. *Parasitology Research*, *118*(3), 1039–1044. https://doi.org/10.1007/s00436-019-06216-x

Zajac, A., Fairman, D., McGee, E., Wells, B., Peregrine, A., Jenkins, E., LeRoith, T., & St John, B. (2020). Alveolar echinococcosis in a dog in the eastern United States. *Journal of Veterinary Diagnostic Investigation*, *32*(5), 742–746. https://doi.org/10.1177/1040638720943842

Zajac, A. M., Conboy, G. A., Little, S. E., & Reichard, M. V. (with American Association of Veterinary Parasitologists). (2021). *Veterinary clinical parasitology* (Ninth edition). Wiley Blackwell.

Zhang, N., Vuppala, N. K., Boney, C. P., Moon, J., Liengswangwong, R., Ahn, H. J. C., Tine, A., & Kendall, T. J. (2024). Primary Pulmonary Echinococcosis in the United States: A Case Report and Review of the Literature. *Cureus*. https://doi.org/10.7759/cureus.55591

Zhu, G.-Q., Li, L., Ohiolei, J. A., Wu, Y.-T., Li, W.-H., Zhang, N.-Z., Fu, B.-Q., Yan, H.-B., & Jia, W.-Z. (2019). A multiplex PCR assay for the simultaneous detection of Taenia hydatigena, T. multiceps, T. pisiformis, and Dipylidium caninum infections. *BMC Infectious Diseases*, *19*(1), 854. https://doi.org/10.1186/s12879-019-4512-3

Zhu, Y., Ye, L., Ding, X., Wu, J., & Chen, Y. (2019). Cerebral sparganosis presenting with atypical postcontrast magnetic resonance imaging findings: A case report and literature review. *BMC Infectious Diseases*, *19*(1), 748. https://doi.org/10.1186/s12879-019-4396-2

Appendix A - Reports of *Echinococcus granulosus* and *E. multilocularis* in domestic and wild canids and humans in the United States

Table 5.1 Reports of *E. granulosus* in domestic and wild canids in the United States, listed by state, definitive host, and disease state, and haplotype in domestic dogs.

State	Disease State & Genotype	Host species	Prevalence (%)	Reference
Alabama				
Alaska	Echinococcosis No genotype specified	Artic foxes (*Alopex lagopus*) Wolf (*Canis lupus*)	65/207 (31.4%)	(R. Rausch & Schiller, 1951; R. Rausch & Williamson, 1959)
	Echinococcosis No genotype specified	Domestic dogs	15/106 (14%)	(R. L. Rausch, 1960)
Arizona	Echinococcosis	Domestic dogs	3/429	(Schantz, Van Alstine, et al., 1977)
	Echinococcosis	Domestic dogs	1/110	(Schantz, von Reyn, et al., 1977)
Arkansas				
California	Echinococcosis	Sheep dogs	7/17 (41.1%)	(Sawyer et al., 1969)
	Echinococcosis	Coyote (*Canis latrans*)	10/213 (4.6%)	(Crellin & Harmon, 1980; I. K. Liu et al., 1970)
Colorado	Echinococcosis	Wolf (*Canis lupus*)	1 animal	(Colorado Conservation Alliance Inc., 2025)
Connecticut				
Delaware				
Florida				
Georgia				
Hawaii				

Idaho	*E. canadensis* (G8/G10)	Gray wolves	43/63 (68.2%)	(Cerda et al., 2018; Foreyt et al., 2009)
Illinois				
Indiana	**Echinococcosis**	**Coyote (*Canis latrans*)**	**1/49 (2%)**	**Our study**
Iowa				
Kansas				
Kentucky	Echinococcosis	Domestic dog	1 case report	(Edney, 1949)
Louisiana	Echinococcosis	Domestic dogs	4 naturally infected	(Beaver, 1954)
Maine	Echinococcosis. *E. canadensis* (G8/10)	Coyotes (*Canis latrans*)	5/23 (21.7%)	(J. M. Schurer et al., 2018)
Maryland				
Massachusetts				
Michigan	Echinococcosis; Cervid strain (G8)	Gray wolf (*Canis lupus*)	1/302 (0.32%)	(Melotti, 2013)
Minnesota				
Mississippi	Echinococcosis; Genotype not specified	Domestic dogs	2/50 (4%)	(Franklin & Ward, 1953)
Missouri				
Montana	Echinococcosis; Genotype not specified	Gray wolf (*Canis lupus*)	38/60 (63%)	(Foreyt et al., 2009)
Nebraska				
Nevada				
New Hampshire				
New Jersey				
New Mexico	*E. granulosus*	Domestic Dogs	6 case reports	(Schantz, Van Alstine, et al., 1977)
	E. granulosus	Domestic dogs	4 case reports	(Schantz, von Reyn, et al., 1977)
New York				
North Carolina				
North Dakota				
Ohio				
Oklahoma				
Oregon				

Pennsylvania	Echinococcosis; *E. canadensis* (G8)	Coyotes (*Canis latrans*)	2/155 (1.29%)	(Garrett, 2021)
Rhode Island				
South Carolina				
South Dakota				
Tennessee	Echinococcosis	Domestic dogs	2/40 case reports	(Edney, 1940)
Texas				
Utah	Echinococcosis;	Domestic Dogs	95/839 (11.3%)	(Loveless et al., 1978)
Vermont				
Virginia				
Washington				
West Virginia				
Wisconsin				
Wyoming	*E. granulousus* sensu and *E. canadensis* (G8/G10)	Gray wolf (*Canis lupus*) Coyote (*Canis latrans*) Red fox (*Vulpes vulpes*)	26/277 (9.3%)	(Brandell et al., 2022; Pipas et al., 2021)

*Shaded states indicate no cases reported of *E. granulosus* in wild or domestic felids.

Table 5.2 Reports of *E. multilocularis* in domestic and wild canids in the United States, listed by state, definitive host, and disease state in domestic dogs.

State	Disease state & Haplotype	Host species	Prevalence (%)	Reference
Alabama				
Alaska	Echinococcosis	Arctic fox (*Vulpes lagopus*) Red fox (*Vulpes vulpes*)	1229/1819 (67.6%)	Rausch, 1956; Rausch and Fay, 2002; Kirk, 2011
Arizona				
Arkansas				
California				
Colorado	Echinococcosis; European haplotype	Domestic dog	2 case reports	(Evason et al., 2025)
Connecticut				
Delaware				
Florida				
Georgia				
Hawaii				

Idaho	Echinococcosis; European haplotype	Domestic dog	1 case report	(Evason et al., 2025)
Illinois	Echinococcosis	Red fox (*Vulpes vulpes*) Coyote (*Canis latrans*)	10/57 (17.5%)	(Ballard & Vande Vusse, 1983; Storandt & Kazacos, 1993)
	Echinococcosis: European haplotype	Domestic dog	3 case reports	(Evason et al., 2025)
	Echinococcosis	**Coyote (*Canis latrans*)**	**10/50 (20%)**	**Our Study**
Indiana	Echinococcosis	Red fox (*Vulpes vulpes*)	1 animal	(Nakao et al., 2009b)
	Echinococcosis	Red fox (*Vulpes vulpes*) Coyote (*Canis latrans*)	29/141 (20.6%)	(Storandt & Kazacos, 1993)
	Echinococcosis	**Coyote (*Canis latrans*)**	**7/49 (14%)**	**Our study**
Iowa	Echinococcosis	Red fox (*Vulpes vulpes*) Coyote (*Canis latrans*)	8/2050 (0.39%)	(Leiby et al., 1970; Redman et al., 2016)
Kansas	Echinococcosis	Red fox (*Vulpes vulpes*) Coyote (*Canis latrans*)	0/111 (0%)	(Storandt et al., 2002)
	Echinococcosis	Domestic dog	1 case report	(Evason et al., 2025)
	Echinococcosis; European haplotype	**Coyote (*Canis latrans*)**	**25/53 (47%)**	**Our Study**
Kentucky	Echinococcosis	Coyote (*Canis latrans*)	1	(Kentucky Fish & Wildlife, 2024)
Louisiana				
Maine				
Maryland				
Massachusetts				
Michigan	Not identified	Coyote (*Canis latrans*)	5/400 (1.3%)	(Melotti, 2013; Storandt & Kazacos, 2012)
Minnesota	Red fox	Red fox (*Vulpes vulpes*)	14/277 (5.0%)	(Leiby et al., 1970)

Mississippi				
Missouri	**Echinococcosis**	**Coyote (*Canis latrans*)**	**22/52 (42%)**	**Our study**
Montana	Echinococcosis	Red fox (*Vulpes vulpes*) Coyote (*Canis latrans*)	9/260 (3.5%)	(Leiby et al., 1970; Seesee et al., 1983)
	Echinococcosis; European haplotype	Domestic dog	2 case reports	(Evason et al., 2025)
Nebraska	Echinococcosis	Red fox (*Vulpes vulpes*) Coyote (*Canis latrans*)	44/168 (26.1%)(Kentucky Fish & Wildlife, 2024)	(Ballard & Vande Vusse, 1983; Redman et al., 2016; Storandt et al., 2002)
Nevada	Echinococcosis; European haplotype	Domestic dog	1 case report	(Evason et al., 2025)
New Hampshire				
New Jersey				
New Mexico				
New York	*E. multilocularis*; Slovakia haplotype	Coyote (*Canis latrans*)	8/96 (8.3%)	(Conlon et al., 2023)
North Carolina				
North Dakota		Red fox (*Vulpes vulpes*) Coyote (*Canis latrans*)	403/2251 (17.9%)	(Kritsky & Leiby, 1978; Leiby et al., 1970; R. L. Rausch & Richards, 1971)
	Echinococcosis; No haplotype identified	Domestic cats	2 cats in one case report	Leiby and Kritsky, 1972
Ohio		Red fox (*Vulpes vulpes*) Coyote (*Canis latrans*)	17/84 (20.2)	(Storandt & Kazacos, 1993, 2012)
Oklahoma				
Oregon	Echinococcosis; European haplotype	Domestic dog	1 case report	(Evason et al., 2025)

Pennsylvania	*E. multilocurlaris*; Haplotype not identifed	Coyote (*Canis latrans*)	2/155 (1.29%)	(Garrett, 2021)
Rhode Island				
South Carolina	*E. multilocularis*; haplotype not identifed	Red fox (*Vulpes vulpes*)	3/44 (6.8%)	Davidson et al., 1992
South Dakota		Red fox (*Vulpes vulpes*) Coyote (*Canis latrans*)	107/397 (27%)	(Hildreth et al., 2000; Leiby et al., 1970)
	Echinococcosis		1 animal	Nakao et al., 2009
Tennessee				
Texas				
Utah				
Vermont				
Virginia	AE; European haplotype	Domestic dog	1 case report	(A. Zajac et al., 2020)
Washington	AE; European haplotype	Domestic dog	4 case reports	(Williams & Walzthoni, 2023)
	Echinococcosis; European haplotype	Domestic dog	4 case reports	(Evason et al., 2025)
West Virginia				
Wisconsin		Red fox (*Vulpes vulpes*) Gray fox (*Urocyon cinereoargenteus*)	6/103 (5.8%)	(Ballard & Vande Vusse, 1983)
Wyoming		Red fox (*Vulpes vulpes*)	0/31 (0%)	(Storandt et al., 2002)
	Echinococcosis	Domestic dog	1 case report	Evason et al., 2024

*Shaded states indicate no cases reported of *E. multilocularis* in wild or domestic felids.

Table 5.3 Reports of *E. granulosus* and *E. multilocularis* in humans in the United States, listed by state, disease state with *E. granulosus strain* and *E. multilocularis* haplotype and speculation of where infection was acquired.

State	*Echinococcus* spp.	Disease	Speculation of infection location	References
Alabama	*E. granulosus* No genotype identified	Pulmonary CE	Immigrated to USA from the Middle East	(Zhang et al., 2024)
Alaska	*E. granulosus* No genotype identified	Pulmonary CE (30 cases)	Alaska	(Pinch & Wilson, 1973)p
	E. granulosus and *E. multilocularis*	41 CE cases 10 AE cases	Alaska	(R. L. Rausch, 1960)
	E. granulosus	101 CE cases	Alaska	(Wilson et al., 1968)
	E. granulosus	33 CE Cases	Alaska	(Wilson & Rausch, 1980)
	E. granulosus	2 CE cases	Washington State	(Castrodale et al., 2002)
Arizona	*E. granulosus*	Hepatic CE	Immigrated to USA from Uzbekistan	(Lloyd et al., 2014)
	E. granulosus	CE in 7 Navajo, 2 Zuni and 2 Domingo Indiana	Locally acquired	(Schantz, von Reyn, et al., 1977)
	E. granulosus	16 CE cases	Arizona and New Mexico	(Schantz et al., 1976)
	E. granulosus	2 Pulmonary CE	Arizona	(R. Katz et al., 1980)
Arkansas	*E. granulosus*	CE	Arkansas	(Narain et al., 1986)
California	*E. granulosus*	CE	Lived in Arizona seven years prior	(Snyder, 1914)
	E. granulosus	Hepatic CE	California	(Lavers, 1957)
	E. granulosus	2 cases of CE	California or Spain	(Sawyer et al., 1969)
	E. granulosus	Pulmonary CE	California	(Schantz et al., 1970)
	E. granulosus	26 cases of Hepatic CE	California	Pitt et al., 1986
	E. granulosus	Hepatic CE	California or Mexico	(Passarelli et al., 2022)
Colorado				
Connecticut	*E. granulosus*	CE	Immigrated from and travels to/from Poland	(Hernandez-Trujillo et al., 2009)
Delaware				

Florida	*E. granulosus*	Pulmonary CE	Moved from California, and stationed in Afghanistan	(Rybolt et al., 2024)
	E. multilocularis	AE	Florida or Albania	(Harper et al., 2020)
	E. multilocularis	Pulmonary AE	Lived in Africa, Asia and Europe	(Harper et al., 2020)
Georgia				
Hawaii				
Idaho				
Illinois	*E. granulosus* and *E. multilocularis*	5 CE cases 2 AE cases	Travel history unknown	(Taxy et al., 2017)
Indiana				
Iowa				
Kansas				
Kentucky	*E. granulosus*	CE	Immigrated to USA from Italy	(Bryan & Schantz, 1989)
Louisiana	*E. granulosus*	Uterine CE	Louisiana	(Miller & Collins, 1937)
	E. granulosus	Hepatic CE	Minnesota or Louisiana	(Sawitz, 1938)
	E. granulosus	Pulmonary CE	Lived in Idaho, Alaska and Louisiana	(Burlew et al., 1990)
Maine	*E. granulosus*	Cerebral CE	Immigrated to USA from Rwanda	(Rebusi, n.d.)
Maryland	*E. granulosus*	Hepatic CE	Immigrated to USA from the Middle East	(Parwani et al., 2004)
Massachusetts	*E. granulosus*	79 cases of CE up to 1948	1 case originated in the USA	(A. M. Katz, 1958)
	E. granulosus	Hepatic and Pulmonary CE	Spent 11 months in Bolivia, 3 years before presentation	(Cabot et al., 1987)
	E. granulosus	Hepatic and Pulmonary CE	Immigrated to USA from Albania	(Baden & Elliott, 2003)
	E. granulosus	Hepatic CE	Immigrated to USA from Romania	(Murali et al., 2015)
Michigan				
Minnesota	*E. multilocularis* North American N2 haplotype	Hepatic AE	Minnesota	(Gamble, 1979; Yamasaki et al., 2008)
Mississippi	*E. granulosus*	Pulmonary CE	Mississippi	(Johnston & Twente, 1952)

Missouri	*E. granulosus*	Pulmonary CE	Immigrated to USA from Israel	(Lev-Tzion & Goldbart, 2012)
Montana				
Nebraska				
Nevada				
New Hampshire	*E. granulosus*	Pulmonary CE	New Hampshire	(AlSalman et al., 2023)
New Jersey	*E. granulosus*	Hepatic CE		(Demos, 1974)
New Mexico	*E. granulosus*	16 CE cases	New Mexico or Arizona	(Schantz et al., 1976)
	E. granulosus	Pulmonary CE	New Mexico or Arizona	(R. Katz et al., 1980)
New York				
North Carolina				
North Dakota				
Ohio	*E. granulosus*	Pulmonary CE	Immigrated to USA from Italy	(G. Thomas et al., 1984)
Oklahoma				
Oregon				
Pennsylvania	*E. granulosus*	Pulmonary CE	Born and raised in Pennsylvania with travel to Palestine	(Blatz et al., 2022)
	E. multilocularis	Hepatic AE	Pennsylvania with a distant travel history to Morocco and recent travel history to Northern Europe	(Komisarof et al., 2000)
Rhode Island				
South Carolina	*E. granulosus*	Hepatic CE	Traveled to Guatemala several years before presentation	(Lin et al., 2024)
South Dakota				
Tennessee	*E. granulosus*	Hepatic CE	Mississippi or Tennessee	(Finck & Hunninen, 1954)
	E. granulosus	Hepatic	Mississippi or Tennessee	(Finck & Hunninen, 1954)
Texas	*E. granulosus*	Pulmonary CE	Spent 3-months in Republic of Georgia and New Mexico, 3 years earlier	(Kornfeld & Mark, 1999)
Utah	*E. granulosus*	56 cases of CE	32 considered autochthonous	Andersen et al., 1977

Vermont	*E. multilocularis* European 2, 4, 5 haplotypes	Hepatic AE and Pulmonary AE	Vermont	(Polish et al., 2021, 2022)
Virginia	*E. granulosus*	Hepatic CE	Immigrated to USA from Afghanistan	(Carter et al., 2009)
Washington				
West Virginia				
Wisconsin				
Wyoming				

*Shaded states indicate no cases reported of *E. granulosus* or *E. multilocularis* in humans.

Appendix B - Museum catalog for host specimens collected in Kansas, Missouri, Indiana, and Illinois, U.S.A.

Table 5.4 Museum catalog for host specimens collected in Kansas listed by catalog number, species, county and gastrointestinal parasites found. To view the full data set, visit Arctos digital specimen database (https://arctos.database.museum/) and enter the catalog number.

Catalog Number	Animal ID	Species	County	Endoparasites found
	C-KS-01	*Canis latrans*	Jefferson	*Taenia spp.*
KSB:Mamm:1589	C-KS-02	*Canis latrans*	Jefferson	***Echinococcus multilocularis***
KSB:Mamm:1596	C-KS-03	*Canis latrans*	Morris	***Echinococcus multilocularis***, *Toxascaris leonina*
KSB:Mamm:1673	C-KS-04	*Canis latrans*	Geary	*Toxascaris leonina*
	C-KS-05	*Canis latrans*	Mitchell	*Toxascaris leonina*
KSB:Mamm:1711	C-KS-06	*Canis latrans*	Ellis	*Toxascaris leonina*
	C-KS-07	*Canis latrans*	Chase	***Echinococcus multilocularis***, *Toxascaris leonina*
	C-KS-08	*Canis latrans*	Sumner	*Taenia spp., Toxascaris leonina*
KSB:Mamm:1571	C-KS-09	*Canis latrans*	Clay	*Physaloptera spp.*
KSB:Mamm:1631	C-KS-10	*Canis latrans*	Morris	*Taenia spp., Physaloptera spp.*
KSB:Mamm:1603	C-KS-11	*Canis latrans*	Jefferson	***Echinococcus multilocularis***, *Taenia spp., Ancylostoma spp.*
KSB:Mamm:1533	C-KS-12	*Canis latrans*	Morris	No parasites found
KSB:Mamm:1277	C-KS-13	*Canis latrans*	Saline	***Echinococcus multilocularis***, *Physaloptera spp.*

KSB:Mamm:1684	C-KS-14	*Canis latrans*	Jefferson	***Echinococcus multilocularis***
KSB:Mamm:1634	C-KS-15	*Canis latrans*	Jefferson	*Toxascaris leonina*
KSB:Mamm:1648	C-KS-16	*Canis latrans*	Jackson	*Ancylostoma spp., Physaloptera spp., Toxascaris spp.*
KSB:Mamm:1602	C-KS-17	*Canis latrans*	Osage	***Echinococcus multilocularis***
KSB:Mamm:1632	C-KS-18	*Canis latrans*	Osage	***Echinococcus multilocularis***
KSB:Mamm:1718	C-KS-19	*Canis latrans*	Osage	***Echinococcus multilocularis***, *Taenia spp., Toxascaris leonina*
KSB:Mamm:1705	C-KS-20	*Canis latrans*	Clay	*Toxascaris leonina*
KSB:Mamm:1594	C-KS-21	*Canis latrans*	Kiowa	*Taenia spp., Physaloptera spp.*
KSB:Mamm:1686	C-KS-22	*Canis latrans*	Ness	*Taenia spp., Toxascaris leonina*
KSB:Mamm:1604	C-KS-23	*Canis latrans*	Rush	*Toxascaris leonina*
KSB:Mamm:1605	C-KS-24	*Canis latrans*	Gray	***Echinococcus multilocularis***, *Taenia spp., Physaloptera spp.*
KSB:Mamm:1569	C-KS-25	*Canis latrans*	Gray	No parasites found
KSB:Mamm:1591	C-KS-26	*Canis latrans*	Kiowa	***Echinococcus multilocularis***, *Taenia spp., Toxascaris leonina*
KSB:Mamm:1288	C-KS-27	*Canis latrans*	Rooks	*Taenia spp., Mesocestoides spp.*
KSB:Mamm:1152	C-KS-28	*Canis latrans*	Rush	*Taenia spp., Toxascaris leonina*
KSB:Mamm:1636	C-KS-29	*Canis latrans*	Rush	*Taenia spp.*
KSB:Mamm:1607	C-KS-30	*Canis latrans*	Rush	No parasites found
KSB:Mamm:1708	C-KS-31	*Canis latrans*	Kiowa	No parasites found

KSB:Mamm:1516	C-KS-32	*Canis latrans*	Gray	*Taenia spp., Physaloptera spp.*
KSB:Mamm:1609	C-KS-33	*Canis latrans*	Rush	No parasites found
KSB:Mamm:1692	C-KS-34	*Canis latrans*	Gray	***Echinococcus multilocularis***
KSB:Mamm:1620	C-KS-35	*Canis latrans*	Neosho	***Echinococcus multilocularis***, *Taenia spp.*
KSB:Mamm:1575	C-KS-36	*Canis latrans*	Lincoln	No parasites found
KSB:Mamm:1691	C-KS-37	*Canis latrans*	-	*Toxascaris leonina*
KSB:Mamm:1695	C-KS-38	*Canis latrans*	Neosho	***Echinococcus multilocularis***
KSB:Mamm:1700	C-KS-39	*Canis latrans*	Neosho	*Toaxascaris leonina*
KSB:Mamm:1622	C-KS-40	*Canis latrans*	Neosho	***Echinococcus multilocularis***, *Toaxascaris leonina*
KSB:Mamm:1685	C-KS-41	*Canis latrans*	Neosho	***Echinococcus multilocularis***
KSB:Mamm:1612	C-KS-42	*Canis latrans*	Neosho	***Echinococcus multilocularis***, *Taenia spp.*
KSB:Mamm:1697	C-KS-43	*Canis latrans*	Neosho	***Echinococcus multilocularis***, *Taenia spp.*
KSB:Mamm:1592	C-KS-44	*Canis latrans*	Neosho	No parasites found
KSB:Mamm:1696	C-KS-45	*Canis latrans*	Neosho	***Echinococcus multilocularis***
KSB:Mamm:1626	C-KS-46	*Canis latrans*	Neosho	***Echinococcus multilocularis***, *Taenia spp., Ancylostoma spp.*
KSB:Mamm:1698	C-KS-47	*Canis latrans*	Neosho	***Echinococcus multilocularis***, *Toxascaris leonina*
KSB:Mamm:1699	C-KS-48	*Canis latrans*	Neosho	*Taenia spp., Toxascaris leonina*
KSB:Mamm:1601	C-KS-49	*Canis latrans*	Thomas	*Taenia spp., Physaloptera spp.*

KSB:Mamm:1590	C-KS-50	*Canis latrans*	Lyon	***Echinococcus multilocularis***, *Taenia spp.*
KSB:Mamm:1647	C-KS-51	*Canis latrans*	Lyon	***Echinococcus multilocularis***, *Taenia spp., Ancylostoma spp.*
KSB:Mamm:1688	C-KS-52	*Canis latrans*	Leavenworth	***Echinococcus multilocularis***, *Taenia spp., Ancylostoma spp., Trichuris spp.*
KSB:Mamm:1608	C-KS-53	*Canis latrans*	Leavenworth	***Echinococcus multilocularis***, *Taenia spp., Toaxacasris leonina*
KSB:Mamm:1531	F-KS-01	*Vulpes vulpes*	McPherson	No parasites found

*Shaded rows had no samples submitted for cataloging.

Table 5.5 Museum catalog for host specimens collected in Missouri listed by catalog number, species, county and gastrointestinal parasites found. To view the full data set, visit Arctos digital specimen database (https://arctos.database.museum/) and enter the catalog number.

Catalog Number	Animal ID	Species	County	Endoparasites found
KSB:Mamm:1198	C-MO-01	*Canis latrans*	Boonville	No parasites found
KSB:Mamm:1611	C-MO-02	*Canis latrans*	Boonville	***Echinococcus multilocularis***, *Taenia spp., Toxascaris leonina*
KSB:Mamm:1490	C-MO-03	*Canis latrans*	Boonville	***Echinococcus multilocularis***, *Taenia spp.*
KSB:Mamm:1694	C-MO-04	*Canis latrans*	Boonville	No parasites found
KSB:Mamm:1252	C-MO-05	*Canis latrans*	Howard	***Echinococcus multilocularis***, *Taenia spp.*
KSB:Mamm:1196	C-MO-06	*Canis latrans*	Howard	*Taenia spp.*
KSB:Mamm:1703	C-MO-07	*Canis latrans*	Howard	*Taenia spp.*
KSB:Mamm:1364	C-MO-08	*Canis latrans*	Howard	***Echinococcus multilocularis***, *Taenia spp.*
KSB:Mamm:1203	C-MO-09	*Canis latrans*	Howard	No parasites found

KSB:Mamm:1630	C-MO-10	*Canis latrans*	Howard	***Echinococcus multilocularis***, *Toxascaris leonina*
KSB:Mamm:1199	C-MO-11	*Canis latrans*	Howard	*Toxascaris leonina*
KSB:Mamm:1193	C-MO-12	*Canis latrans*	Putnam	*Taenia spp.*
KSB:Mamm:1606	C-MO-13	*Canis latrans*	Putnam	*Taenia spp.*
KSB:Mamm:1197	C-MO-14	*Canis latrans*	Mercer & Grundy	***Echinococcus multilocularis***
KSB:Mamm:1228	C-MO-15	*Canis latrans*	Mercer & Grundy	***Echinococcus multilocularis***
KSB:Mamm:1323	C-MO-16	*Canis latrans*	Atchinson & Holt	***Echinococcus multilocularis***, *Physaloptera spp.*
KSB:Mamm:1160	C-MO-17	*Canis latrans*	Atchinson & Holt	No parasites found
KSB:Mamm:1194	C-MO-18	*Canis latrans*	Platte	***Echinococcus multilocularis***
KSB:Mamm:1188	C-MO-19	*Canis latrans*	Platte	***Echinococcus multilocularis***, *Taenia spp., Toxascaris leonina*
KSB:Mamm:1689	C-MO-20	*Canis latrans*	Dekalb & Gentry	***Echinococcus multilocularis***, *Ancylostoma spp.*
KSB:Mamm:1229	C-MO-21	*Canis latrans*	Sullivan	No parasites found
KSB:Mamm:1141	C-MO-22	*Canis latrans*	Sullivan	*Taenia spp.*
KSB:Mamm:1666	C-MO-23	*Canis latrans*	Putnam	*Taenia spp.*
KSB:Mamm:1670	C-MO-24	*Canis latrans*	Putnam	*Taenia spp., Toxascaris leonina*
KSB:Mamm:1195	C-MO-25	*Canis latrans*	Atchinson & Holt	***Echinococcus multilocularis***, *Taenia spp., Physaloptera spp.*
KSB:Mamm:1334	C-MO-26	*Canis latrans*	Atchinson & Holt	*Ancylostoma spp.*
KSB:Mamm:1221	C-MO-27	*Canis latrans*	Mercer & Grundy	*Taenia spp.*

KSB:Mamm:1624	C-MO-28	*Canis latrans*	Mercer & Grundy	*Taenia spp., Physaloptera spp,*
KSB:Mamm:1225	C-MO-29	*Canis latrans*	Harrison	*Taenia spp., Physaloptera spp,*
KSB:Mamm:1230	C-MO-30	*Canis latrans*	Harrison	No parasites seen
KSB:Mamm:1226	C-MO-31	*Canis latrans*	Dekalb & Gentry	*Taenia spp., Toxascaris leonina*
KSB:Mamm:1231	C-MO-32	*Canis latrans*	Dekalb & Gentry	***Echinococcus multilocularis***, *Taenia spp.*
KSB:Mamm:1677	C-MO-33	*Canis latrans*	Atchinson & Holt	*Toxascaris leonina*
KSB:Mamm:1637	C-MO-34	*Canis latrans*	Cole	*Taenia spp., Physaloptera spp.*
KSB:Mamm:1524	C-MO-35	*Canis latrans*	Howard	***Echinococcus multilocularis***, *Dirofilaria immitis*
KSB:Mamm:1671	C-MO-36	*Canis latrans*	Howard	No parasites found
KSB:Mamm:1709	C-MO-37	*Canis latrans*	Howard	*Toxascaris leonina*
KSB:Mamm:1599	C-MO-38	*Canis latrans*	Howard	***Echinococcus multilocularis***, *Dirofilaria immitis*
KSB:Mamm:1706	C-MO-39	*Canis latrans*	Howard	No parasites found
KSB:Mamm:1597	C-MO-40	*Canis latrans*	Howard	*Ancylostoma spp., Dirofilaria immitis*
KSB:Mamm:1149	C-MO-41	*Canis latrans*	Dade	*Taenia spp., Toxascaris leonina*
KSB:Mamm:1627	C-MO-42	*Canis latrans*	Cass	***Echinococcus multilocularis***
KSB:Mamm:1528	C-MO-43	*Canis latrans*	Polk	***Echinococcus multilocularis***, *Taenia spp.*
KSB:Mamm:1579	C-MO-44	*Canis latrans*	Lawrence	*Taenia spp., Toxascaris leonina*
KSB:Mamm:1600	C-MO-45	*Canis latrans*	Lawrence	***Echinococcus multilocularis***, *Physaloptera spp.*

KSB:Mamm:1587	C-MO-46	*Canis latrans*	Barton	*Taenia spp.*
KSB:Mamm:1227	C-MO-47	*Canis latrans*	Barton	***Echinococcus multilocularis***
KSB:Mamm:1598	C-MO-48	*Canis latrans*	Barton	***Echinococcus multilocularis***, *Taenia spp.*
KSB:Mamm:1159	C-MO-49	*Canis latrans*	McDonald	*Taenia spp.*
KSB:Mamm:1588	C-MO-50	*Canis latrans*	McDonald	*Ancylostoma spp.*
KSB:Mamm:1690	C-MO-51	*Canis latrans*	Camden	***Echinococcus multilocularis***, *Toxascaris leonina*
KSB:Mamm:1610	C-MO-52	*Canis latrans*	Camden	***Echinococcus multilocularis***
KSB:Mamm:1707	F-MO-01	*Canis latrans*	Jackson	***Echinococcus multilocularis***

*Shaded rows had no samples submitted for cataloging.

Table 5.6 Museum catalog for host specimens collected in Illinois listed by catalog number, species, county and gastrointestinal parasites found. To view the full data set, visit Arctos digital specimen database (https://arctos.database.museum/) and enter the catalog number.

Catalog Number	Animal ID	Species	County	Endoparasites found
KSB:Mamm:1674	C-IL-01	*Canis latrans*	Macoupin/Fayette	***Echinococcus multilocularis***, *Ancylostoma spp.*
KSB:Mamm:1633	C-IL-02	*Canis latrans*	Macoupin/Fayette	*Taenia spp.*
KSB:Mamm:1641	C-IL-03	*Canis latrans*	Macoupin/Fayette	***Echinococcus multilocularis***, *Taenia spp.*
KSB:Mamm:1715	C-IL-04	*Canis latrans*	Macoupin/Fayette	*Taenia spp.*
KSB:Mamm:1567	C-IL-05	*Canis latrans*	Macoupin/Fayette	*Ancylostoma spp.*
KSB:Mamm:1644	C-IL-06	*Canis latrans*	Macoupin/Fayette	*Taenia spp.*
KSB:Mamm:1643	C-IL-07	*Canis latrans*	Macoupin/Fayette	*Taenia spp., Ancylostoma spp., Toxascaris leonina*

KSB:Mamm:1720	C-IL-08	*Canis latrans*	Christian	***Echinococcus multilocularis***, *Ancylostoma spp., Toxascaris leonina*
	C-IL-09	*Canis latrans*	Christian	***Echinococcus multilocularis***, *Taenia spp., Toxascaris leonina*
KSB:Mamm:1668	C-IL-10	*Canis latrans*	Christian	*Taenia spp., Ancylostoma spp., Toxascaris spp. Trichuris spp.*
KSB:Mamm:1651	C-IL-11	*Canis latrans*	Christian	***Echinococcus multilocularis***, *Taenia spp., Ancylostoma spp.. Physaloptera spp., Toxascaris spp.*
KSB:Mamm:1539	C-IL-12	*Canis latrans*	Christian	No parasites found
KSB:Mamm:1649	C-IL-13	*Canis latrans*	Christian	*Taenia spp., Ancylostoma spp.*
KSB:Mamm:1570	C-IL-14	*Canis latrans*	Christian	*Taenia spp., Ancylostoma spp., Physaloptera spp., Toxascaris spp.*
KSB:Mamm:1655	C-IL-15	*Canis latrans*	Christian	*Taenia spp., Physaloptera spp.*
KSB:Mamm:1669	C-IL-16	*Canis latrans*	Christian	*Taenia spp., Ancylostoma spp., Tocascaris leonina*
KSB:Mamm:1658	C-IL-17	*Canis latrans*	Henry	*Ancylostoma spp., Physaloptera spp.*
KSB:Mamm:1664	C-IL-18	*Canis latrans*	Rock Island	*Taenia spp.*
KSB:Mamm:1638	C-IL-19	*Canis latrans*	Knox/Henry/Rock Island	*Taenia spp., Ancylostoma spp., Physaloptera spp.*
KSB:Mamm:1513	C-IL-20	*Canis latrans*	Bureau/La Salle	*Taenia spp., Ancylostoma spp., Toxascaris leonina*
KSB:Mamm:1522	C-IL-21	*Canis latrans*	Rock Island	*Taenia spp., Ancylostoma spp.*
KSB:Mamm:1635	C-IL-22	*Canis latrans*	Rock Island	*Taenia spp.*
KSB:Mamm:1511	C-IL-23	*Canis latrans*	Henry	*Taenia spp., Physaloptera spp.*
KSB:Mamm:1657	C-IL-24	*Canis latrans*	Henry	*Ancyslostoma spp., Toxascaris leonina*

KSB:Mamm:1656	C-IL-25	*Canis latrans*	Bureau/La Salle	*Taenia spp., Dirofilaria immitis, Toxascaris leonina*
KSB:Mamm:1527	C-IL-26	*Canis latrans*	Whiteside	*Taenia spp., Ancylostoma spp.*
KSB:Mamm:1537	C-IL-27	*Canis latrans*	Rock Island	*Taenia spp., Toxascaris leonina*
KSB:Mamm:1663	C-IL-28	*Canis latrans*	Rock Island	*Taenia spp., Ancylostoma spp,*
KSB:Mamm:1650	C-IL-29	*Canis latrans*	Bureau/La Salle	***Echinococcus multilocularis***, *Taenia spp., Toxascaris spp,*
KSB:Mamm:1544	C-IL-30	*Canis latrans*	Whiteside	*Ancylostoma spp. Toxascaris leonina*
KSB:Mamm:1549	C-IL-31	*Canis latrans*	Bureau/La Salle	*Taenia spp., Physaloptera spp., Toxascaris leonina*
KSB:Mamm:1667	C-IL-32	*Canis latrans*	Warren	*Taenia spp., Ancylostoma spp., Toxascaris leonina*
KSB:Mamm:1672	C-IL-33	*Canis latrans*	Warren	*Ancylostoma spp.*
KSB:Mamm:1659	C-IL-34	*Canis latrans*	Whiteside	*Ancylostoma spp., Physaloptera spp., Toxascaris leonina*
KSB:Mamm:1645	C-IL-35	*Canis latrans*	Whiteside	*Taenia spp., Ancylostoma spp.*
KSB:Mamm:1525	C-IL-36	*Canis latrans*	Knox/Henry/Rock Island	***Echinococcus multilocularis***, *Ancylostoma spp., Physaloptera spp.*
KSB:Mamm:1642	C-IL-37	*Canis latrans*	Henry	*Taenia spp., Ancylostoma spp. & Toxascaris spp.*
KSB:Mamm:1565	C-IL-38	*Canis latrans*	Henry	***Echinococcus multilocularis***, *Taenia spp., Ancylostoma spp. & Physaloptera spp.*
KSB:Mamm:1572	C-IL-39	*Canis latrans*	Whiteside	*Taenia spp., Ancylostoma spp., Physaloptera spp., Toxascaris spp.*
KSB:Mamm:1577	C-IL-40	*Canis latrans*	Bureau/La Salle	*Taenia spp., Ancylostoma spp., Physaloptera spp.*
KSB:Mamm:1662	C-IL-41	*Canis latrans*	Rock Island	*Taenia spp.*

KSB:Mamm:1526	C-IL-42	*Canis latrans*	Whiteside	*Toxascaris spp.*
KSB:Mamm:1584	C-IL-43	*Canis latrans*	Henry	*Taenia spp.*, *Ancylostoma spp.*
KSB:Mamm:1581	C-IL-44	*Canis latrans*	Whiteside	*Taenia spp.*, *Ancylostoma spp.*
KSB:Mamm:1580	C-IL-45	*Canis latrans*	Henry	***Echinococcus multilocularis***, *Taenia spp.*, *Ancylostoma spp.*, *Physaloptera spp.*, *Toxascaris leonina*
KSB:Mamm:1621	C-IL-46	*Canis latrans*	Rock Island	*Taenia spp.*, *Ancylostoma spp.* & *Toxascaris leonina*
KSB:Mamm:1583	C-IL-47	*Canis latrans*	Henry	*Taenia spp.*, *Physaloptera spp.*
KSB:Mamm:1578	C-IL-48	*Canis latrans*	Knox/Henry/Rock Island	***Echinococcus multilocularis***, *Taenia spp.*
KSB:Mamm:1710	C-IL-49	*Canis latrans*	Whiteside	No parasites found
KSB:Mamm:1573	C-IL-50	*Canis latrans*	Rock Island	*Taenia spp.*

*Shaded rows had no samples submitted for cataloging.

Table 5.7 Museum catalog for host specimens collected in Indiana listed by catalog number, species, county and gastrointestinal parasites found. To view the full data set, visit Arctos digital specimen database (https://arctos.database.museum/) and enter the catalog number.

Catalog Number	Animal ID	Species	County	Endoparasites found
	C-IN-01	*Canis latrans*	Greene	*Taenia spp.*
KSB:Mamm:1515	C-IN-02	*Canis latrans*	Davies	***Echinococcus multilocularis***, *Taenia spp.*, *Dirofilaria immitis*
KSB:Mamm:1701	C-IN-03	*Canis latrans*	Kokomo	*Physaloptera spp.*
KSB:Mamm:1523	C-IN-04	*Canis latrans*	Selma	***Echinococcus multilocularis***, *Taenia spp.*, *Toxascaris leonina*
KSB:Mamm:1518	C-IN-05	*Canis latrans*	Selma	*Taenia spp.*, *Toxascaris leonina*

KSB:Mamm:1534	C-IN-06	*Canis latrans*	Selma	*Physaloptera spp.*
KSB:Mamm:1553	C-IN-07	*Canis latrans*	Selma	*Taenia spp., Toxascaris leonina*
KSB:Mamm:1542	C-IN-08	*Canis latrans*	Selma	*Taenia spp., Toxascaris leonina*
KSB:Mamm:1547	C-IN-09	*Canis latrans*	Selma	*Taenia spp., Physaloptera spp.*
KSB:Mamm:1512	C-IN-10	*Canis latrans*	Selma	*Taenia spp.*
KSB:Mamm:1514	C-IN-11	*Canis latrans*	Greene	*Ancylostoma spp., Physaloptera spp.*
KSB:Mamm:1551	C-IN-12	*Canis latrans*	Greene	*Dirofilaria immitis*
KSB:Mamm:1564	C-IN-13	*Canis latrans*	Greene	*Toxascaris leonina*
KSB:Mamm:1543	C-IN-14	*Canis latrans*	Greene	*Taenia spp., Toxascaris leonina, Trichuris spp.*
KSB:Mamm:1554	C-IN-15	*Canis latrans*	Greene	*Taenia spp., Toxascaris leonina*
	C-IN-16	*Canis latrans*	Greene	*Taenia spp., Dirofilaria immitis, Toxascaris leonina*
KSB:Mamm:1532	C-IN-17	*Canis latrans*	Greene	***Echinococcus multilocularis***, *Taenia spp., Physaloptera spp.*
KSB:Mamm:1552	C-IN-18	*Canis latrans*	Greene	*Ancylostoma spp., Toxascaris leonina*
KSB:Mamm:1558	C-IN-19	*Canis latrans*	Greene	*Dirofilaria immitis*
KSB:Mamm:1521	C-IN-20	*Canis latrans*	Greene	***Echinococcus multilocularis***, *Ancylostoma spp., Physaloptera spp., Trichuris spp.*
KSB:Mamm:1530	C-IN-21	*Canis latrans*	Greene	*Taenia spp.*
KSB:Mamm:1541	C-IN-22	*Canis latrans*	Greene	*Taenia spp., Dirofilaria immitis, Physaloptera spp., Toxascaris leonina, Trichuris spp.*
KSB:Mamm:1517	C-IN-23	*Canis latrans*	Greene	*Taenia spp., Physaloptera spp., Trichuris spp.*

KSB:Mamm:1535	C-IN-24	*Canis latrans*	Greene	*Taenia spp.*
KSB:Mamm:1536	C-IN-25	*Canis latrans*	Greene	*Dirofilaria immitis*
KSB:Mamm:1510	C-IN-26	*Canis latrans*	Greene	No parasites found
KSB:Mamm:1550	C-IN-27	*Canis latrans*	Greene	No parasites found
KSB:Mamm:1546	C-IN-28	*Canis latrans*	Greene	*Taenia spp.*
KSB:Mamm:1529	C-IN-29	*Canis latrans*	Greene	*Taenia spp., Physaloptera spp.*
KSB:Mamm:1538	C-IN-30	*Canis latrans*	Greene	*Taenia spp., Ancylostoma spp., Toxascaris leonina*
KSB:Mamm:1623	C-IN-31	*Canis latrans*	Kokomo	*Taenia spp.*
KSB:Mamm:1582	C-IN-32	*Canis latrans*	Kokomo	***Echinococcus multilocularis***, *Physaloptera spp.*
	C-IN-33	*Canis latrans*	Kokomo	*Dirofilaria immitis, Physaloptera spp.*
	C-IN-34	*Canis latrans*	Kokomo	No parasites found
KSB:Mamm:1561	C-IN-35	*Canis latrans*	Kokomo	*Taenia spp.*
KSB:Mamm:1576	C-IN-36	*Canis latrans*	Kokomo	*Taenia spp.*
KSB:Mamm:1713	C-IN-37	*Canis latrans*	Kokomo	***Echinococcus multilocularis***, *Taenia spp., Physaloptera spp.*
KSB:Mamm:1625	C-IN-38	*Canis latrans*	Kokomo	***Echinococcus multilocularis***, *Physaloptera spp.*
KSB:Mamm:1563	C-IN-39	*Canis latrans*	Selma	*Taenia spp.*
KSB:Mamm:1559	C-IN-40	*Canis latrans*	Selma	*Taenia spp.*
KSB:Mamm:1556	C-IN-41	*Canis latrans*	Selma	*Taenia spp.*

KSB:Mamm:1560	C-IN-42	*Canis latrans*	Selma	*Taenia spp.*
KSB:Mamm:1723	C-IN-43	*Canis latrans*	Selma	*Taenia spp.*
KSB:Mamm:1562	C-IN-44	*Canis latrans*	Selma	*Taenia spp.*
KSB:Mamm:1661	C-IN-45	*Canis latrans*	Selma	***Echinococcus multilocularis***, *Ancylostoma spp., Physaloptera spp., Toxascaris leonina*
KSB:Mamm:1555	C-IN-46	*Canis latrans*	Selma	*Taenia spp., Dirofilaria immitis*
KSB:Mamm:1540	C-IN-47	*Canis latrans*	Selma	*Taenia spp., Ancylostoma spp., Physaloptera spp., Toxascaris leonina*
KSB:Mamm:1545	C-IN-48	*Canis latrans*	Selma	*Taenia spp., Toxascaris leonina*
KSB:Mamm:1519	C-IN-49	*Canis latrans*	Selma	*Ancylostoma spp., Toxascaris leonina*

*Shaded rows had no samples submitted for cataloging.

Appendix C - GenBank References for *Echinococcus multilocularis* haplotypes

Table 5.8 Haplotypes and corresponding GenBank accession numbers for the *nad2* and *cob* genes.

Echinococcus multilocularis Haplotype and Outgroup	nad2	cob	References
Austria (E1)	AB461403	AB461395	Nakao et al., 2009
Canada: Alberta Haplotype AB1	KC582634	KC582620	Gesy and Jenkins (2015)
Canada: British Columbia isolate BC1	KC550005	KC550003	Gesy and Jenkins (2015)
Canada: British Columbia isolate MC3	JF751036	JF751035	Jenkins et al. (2012)
Canada: Saskatchewan haplotype SK1	KC550008	KC550006	Gesy and Jenkins (2015)
Canada: Saskatchewan haplotype SK2	KC582628	KC582614	Gesy and Jenkins (2015)
Canada: Saskatchewan haplotype SK3	KC549993	KC549999	Gesy and Jenkins (2015)
Canada: Saskatchewan haplotype SK4	KC582629	KC582615	Gesy and Jenkins (2015)
Canada: Saskatchewan haplotype SK5	KC582630	KC582616	Gesy and Jenkins (2015)
Canada: Saskatchewan haplotype SK6	KC582631	KC582617	Gesy and Jenkins (2015)
Canada: Saskatchewan haplotype SK7	KC582632	KC582618	Gesy and Jenkins (2015)
Canada: Saskatchewan haplotype SK8	KC582633	KC582619	Gesy and Jenkins (2015)
China: Inner Mongolia (O1)	AB461411	AB461402	Nakao et al. (2009)
France (E2, E3, E4)	AB461404	AB461396	Nakao et al. (2009)
Japan: Hokkaido (A3, A4)	AB461407	AB461399	Nakao et al. (2009)
Kyrgyzstan isolate (A19)	MN829521	MN829505	(Alvarez Rojas et al., 2020)
Poland haplotype EmPL6	KY205697	KY205667	(Karamon et al., 2017)

Poland haplotype EmPL10	KY205701	KY205671	(Karamon et al., 2017)
Poland haplotype EmPL14	KY205705	KY205675	(Karamon et al., 2017)
Slovakia (E5)	AB461405	AB461397	Nakao et al. (2009)
United States: Alaska, St. Lawrence Island (N1)	AB461409	AB461400	Nakao et al. (2009)
United States: Alaska, St. Lawrence Island Isolate A1	MT429275	MT429271	(Laurimäe et al., 2020)
United States: Indiana (N2)	AB461410	AB461401	Nakao et al. (2009)
United States: Missouri	LC645086	LC645085	Kuroki et al. (2022)
United States: Missouri	LC380930	LC380929	**(Kuroki et al., 2020)**
United States: South Dakota (N2)	AB374427	AB374426	Nakao et al. (2009)
United States: Virginia isolate 197WR	OK268249	OK268251	Polish et al. (2022)
United States: New York Isolate E316	OP596325	OP596328	Conlon et al. (2024)
United States: New York Isolate E320	OP596326	OP596329	Conlon et al. (2024)
United States: New York Isolate E321	OP596327	OP596330	Conlon et al. (2024)
Taenia saginata	NC_009938	NC_009938	(Jeon et al., 2007)