Abstract

Intestinal Helminths Of The Wisconsin Muskrat (*Odontra zibethicus*)

*Ondatra zibethicus*, also known as the muskrat, is a semiaquatic rodent commonly found in North America. These rodents contribute significantly to the local economy, environment, and public health. Every year, from October to March, trappers capture these rodentss in Horicon Marsh located in Dodge County, Wisconsin. Once caught, the animals are skinned, and the pelts are sold. The role of this rodent, as a bioindicator, cannot be ignored. Due to its sensitivity to change, the muskrat population can be viewed as an alert system for a deteriorating ecosystem. Several environmental conditions afflicting *Ondatra zibethicus* include drought, anthropogenic contamination, and predation. Most importantly, their ability to be a host for a variety of diseases makes the fur bearer a useful model. In collaboration with trappers at Horicon Marsh, the carcasses of 45 muskrats were examined in this two-part study. Thirty-six carcasses were collected by Sarah Woody, a previous graduate student at UWO, and necropsied in May of 2021. In January 2023 nine more were collected for the same purpose. The tongues retrieved from the muskrats were assayed for the presence of helminths. *Trichinella spp.* is an intracellular nematode that can encyst in the muscle of many hosts including mammals, birds and reptiles. In order to establish their presence within a muskrat, the tongues collected were digested with the help of artificial digestive fluids concocted using HCL and Pepsin. The tongues were cut into pieces, blended, and the mixture observed under a dissecting microscope for the presence of *Trichinella spp.* The small and large intestines were assayed all parasites recovered from the small and large intestines were placed in 80% ethanol. Thereafter, the collected parasites were separated based on visual differences, fixed in formaldehyde, mounted on slides, and morphologically identified. Morphologic identification revealed the presence of intestinal helminths such as *Plagiorchis proximus*, *Quienquiseriales quienquiserialis*, *Echinostoma spp.*, *Trichuris opaca*, and a Cestode *spp.* Mitochondrial and Ribosomal DNA was extracted, and the genetic samples were sent out for sequencing via third party to confirm morphologic identification.
Intestinal Helminths Of The Wisconsin Muskrat (*Ondatra zibethicus*)

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Although I know it is lacking, this is my love letter to all of the strong, resilient, women in my life. To my mentor Dr. Michalski, to my committee member Misty McPhee, to my sisters who I love dearly. To my nieces, cousins, friends, and most importantly – My mother. Mamá, si se pudo.
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Chapter I: Introduction

The Marsh

Horicon Marsh is located on 13,200 hectares on what used to be a glacial lake left behind during the Pleistocene era (Droste, 2015). The lake was ultimately drained via the Rock River, leaving behind mineral deposits like silt and clay which set the foundation for the marsh. The species richness found in the area attracted many nomadic hunters, Native American populations, and European settlers. The evidence left behind, in the form of artifacts, suggests that people inhabited the marsh as far back as 12,000 years ago (Droste, 2015). This amazing treasure in East Central Wisconsin was once home to mammoths, mastodons, and even large ground sloths. Now, the marsh is home to many mammal species, and is an important geographical location for migratory birds.

Muskrat ecology

The muskrat, *Ondatra zibethicus*, is a widely distributed furbearer in North America. The muskrat distribution extends from northern Mexico to Mississippi and Louisiana, continuing all the way to northern Alaska and into parts of northern Canada (Zabiega, 1996). There are many aquatic ecosystems from which the rodent can choose, ranging from streams to lakes, to wetlands or swamps, and even drainage ditches. It occupies almost every type of freshwater aquatic habitat. In these habitats the muskrats are the dominant herbivore, often restructuring landscapes by keeping invasive plant species in order. This rodent can have positive implications for the bird species in an aquatic habitat like a marsh. When these aquatic habitats form dense vegetation, the muskrat will ensure
that the overgrown plants are cut down. The clearings made by the rodents will ensure
water fowl have a place to land in spring making these spaces perfect breeding grounds
(Bishop et al., 1979; Roberts & Crimmins, 2010). They feed on exotic species, like the
*Typha x glauca* cattail which can be found in Horicon Marsh and comprises
approximately 95% of the marsh cattail species (Woody, 2022). Zebra mussels
(*Dreissena polymorpha*), also an exotic species, and a problem within many fresh water
ecosystems are eaten by the rodent. Lastly, muskrats can also serve as indicators of a
healthy ecosystems due to their response to contamination caused by toxins and
chemicals. It is important to venture further into the challenges faced by muskrats in its
relatively short life span to better understand why the species is on the decline. Not only
should we continue to understand their interaction with the environment, but how the
changing environment affects them and to what extent. It is important to look not only at
their surroundings, but to physically look within.

The common muskrat, is a semi aquatic rodent that can be found in many North
American waterways. Although the rodent thrives in many different habitats it prefers
lentic waters (Proulx & Gilbert, 1983). The muskrat is a source of food for many
organisms and also represents a source of income for many Wisconsinites who enjoy
trapping for the fur trade. The muskrat is adapted to life in water, it possesses beaver-like
teeth that are used for a variety of tasks. It uses them for cutting through cattails and
chewing through ice to get to vegetation during the winter months. This is important
because the cattail is an important part of the muskrat diet. Other commonly eaten plants
include: bugleweed, Manna grass, spatterdock, and pondweed (Hamerstrom et al., 1939).
During the summer months the water lily is an important source of nutrients for the muskrat. Although the rodent primarily consumes vegetation, it is not its sole sustenance. They have been known to eat fish, frogs, snails and mussels (Detwiler et al., 2012; Errington, 1941). At times, muskrats will even consume aquatic insects (Maryland Mammals, 2023). Conditions of when they choose these food items over a plant-based diet is not known. Lastly, muskrats have been known to partake in cannibalism. The carcasses of muskrats are eaten by other muskrats, as well as those of kits who were neglected by their parents (Errington, 1941).

The muskrat has a relatively short lifespan of only two years. They mature quickly and are able to have multiple litters. A sexually mature adult can breed up to three times during a season. The mating begins in early spring and the first litter arrives only 30 days after. They have a very short gestation period, and they will continue to breed into early summer. At Horicon Marsh, the average size of a litter is seven with the young muskrat being referred to as a kit. The kits will live with their parents up until six weeks when they are fully mature adults (Droste, 2015). Once those six weeks are up, the kits will leave the lodges and will be off to fend for themselves.
The young offspring will face a variety of predators and are especially vulnerable if they choose to run over frozen waters. Predators of the muskrat includes the *Mustela lutreola, Vulpes vulpes, Canis latrans*, and a variety of other terrestrial mammals (Droste, 2015). Aerial predators like *Strix* spp., *Accipiter* spp., and *Haliaeetus leucocephalus* hunt the rodent. Although there are plenty of organisms that feed on the muskrat, the two main predatory threats to the muskrat are *Mustela lutreola* and *Lutrinae* spp. Otters will often dig through the walls of the lodges so that they have direct access to the muskrats within the lodge. This pattern of predation by *Mustela lutreola* and *Lutrinae* spp. could be location specific or increased under certain environmental circumstances. A study by Wilson surveyed the intestinal contents of 568 digestive tracts of minks and otters in North Carolina. Fifty-three mink scat samples indicated that muskrat was part of the mink...
diet, but mostly during the winter months. The otter on the other hand had no traces of muskrat in any intestinal tract content or in scat samples (Wilson, 1954).

**Muskrat monetary Importance**

The beginning of American fur trading can be traced back to the 1500s when the Frenchman Samuel De Champlin built the very first fur outpost in Quebec, Canada (Droste, 2015). The search for fur bearing animals brought European settlers west into Wisconsin in search for pelt. At this time, coats and hats with fur lining were very popular. Because of this, the muskrat holds monetary importance, especially in Wisconsin. From 1948 to 1964, more than 8.4 million muskrats were harvested for their fur (Mathiak, 1966). Trappers were able to sell the furs for several dollars a pelt, making it a very lucrative business. Some individuals in the state went as far as to buy marsh land for the sole purpose of harvesting muskrats. The demand was so high that most individuals were able to recover any investment made in a short one to two years. As lucrative as this business was, the number of individuals trapping has decreased. The market at times has become unstable and one can see the decrease in people willing to participate. From 1947 to 1967 the price per pelt fluctuated from as high as 34 dollars to under 5 dollars per pelt. The number of harvested muskrats hit a low around 1962 when the price of pelts was under 5 dollars (Roberts & Crimmins, 2010).

The process of acquiring a permit in itself can be costly. Trappers have to bid for the land they want to harvest. Many of these permits can go for up to $1,000. The land is often the initial cost. The trappers must them obtain all of the equipment needed to trap the muskrat. All of the accumulated costs have prevented new individuals from becoming
trappers. Between 1963 and 1967, a full-time trapper in Canada made $6,296 Canadian dollars. That would roughly be $4,655.26 US dollars (Usher, 1965). Using the Bureau of Labor Statistics CPI inflation calculator, that translates to approximately $46,811.55 a year. However, Daigle et al. (1998) says that by 1993 the average trapper was bringing home $434 dollars. Only 15% percent of trappers during this time critically relied on trapping as their main source of income. For the other 85%, other factors contributed to their continual participation. First, they valued the lifestyle. Trapping was viewed as an activity and they enjoyed the art of trapping. Second, through trapping they were able to appreciate nature. Lastly, individuals felt that they could help in the reduction in spread of zoonotic illnesses or help with public health (Daigle et al., 1998).

**Disease**

Many rodents, like *Ondatra zibethicus*, are host species for many bacterial, parasitic and viral pathogens. Bacteria associated with the muskrat include *Francisella tularensis*, *C. piliformis*, and *Staphylococcus* spp. among others. Three fungi species have been found in North American muskrats (*Emmonsia crescens, Encephalitozoon cuniculi, Trichophyton mentagrophytes*) (Ganoe et al., 2020). Cyanobacteria can produce toxins that are a health hazards to birds, mammals, amphibians, and fish. Muskrat death has been reported mostly due to the cyanobacteria *A. flos-aquae* (Ganoe et al., 2020).

Two bacterial infections were of significant concern, namely *Francisella tularensis* and *Clostridium piliforme*. The muskrat harbor’s both of these bacteria which is not only deadly for rodents but transmissible to humans. In humans, *Francisella tularensis* causes tularemia and comes with a variety of symptoms depending on how the bacterium enters
the body. The most common way to acquire the infection is through a bite from a tick or a deer fly while handling an infected individual. This form is called ulceroglandular tularemia and comes with the manifestation of an ulcer and swelling of lymph nodes, usually around the underarms and the groin. If an ulcer is not present, but swollen lymph nodes occur, this is glandular tularemia. Pneumonic tularemia is the more serious of the classifications and presents itself with many symptoms. Those symptoms include chest pain, difficulty breathing, and a cough. According to the CDC, from 1990 to 2000 there were a total of 1,368 cases reported in the United States. Over half of those cases were reported from four states; Arkansas, Missouri, South Dakota, and Oklahoma (Tularemia -- United States, 1990--2000, 2002). Between the years 2001 and 2010, the cases did subtly decrease to 1,208 infected individuals. Missouri, Arkansas, Oklahoma, and South Dakota still contributed to over half of cases. Massachusetts and Kansas saw an increase in the cases reported during the early 2000s (Nelson et al., 2013).

*Clostridium piliforme* causes Tyzzers’s disease in muskrats. The mode of infection is yet to be officially described. However, *C. piliforme* is a spore forming bacteria, and it is believed that the spores are ingested via contact with feces from horses. The spores will travel through the body and into the intestines and liver. In the liver it will cause acute necrotizing hepatitis and will lead to hepatic failure. This bacterium is deadly in muskrats (Karstad et al. 1971). Twenty-two muskrats were caught between October 10 and 17, 1969. Of 22 muskrats caught in Ontario Canada, the first muskrat died on October 23, and four days later four more muskrats had succumbed to the disease. By November 6 four more muskrats had fallen to the illness, and by the end of the outbreak only six of
the 22 muskrats had survived. When the muskrats were necropsied to record the pathology of the disease, they found yellow lesions on the surface of the liver. The researchers observed hemorrhaging of the intestinal wall accompanied by patches of necrotic mucosa in the cecum and colon. In some muskrats there was evidence of bloody discharge coming from the rectum. Humans can also develop Tyzzer’s disease. The condition is very rare and there has only been one confirmed case from an immunosuppressed individual in 1996. The patient came in for medical observation and demonstrated crusted wart-like lesions. RNA sequencing was used to diagnose the patient with a Tyzzer’s infection. The pathology and mode of infection in humans needs to be further investigated. The aforementioned pathogens were on a microscopic level. There are, however, other organisms that are potentially harmful but are often macroscopic. Intestinal parasite of the muskrat have been widely researched, but in Wisconsin not much is known on the composition of the parasitic communities within the intestinal tracts of muskrats.

**Rationale of project**

The project was suggested due to the limited information available on the prevalence and distribution of *Trichinella* and intestinal parasites in the state of Wisconsin. For trichinella, much of the information available comes from European sources. Even less information is available about trichinella in the state of Wisconsin. Before the year 2008, 10% of reported *Trichinella* infection in the US were due to the ingestion of trichinous meat. In the following four years, 49% of all human *Trichinella* infections were related to bear meat consumption. In the United States, from 2008 to 2012 a total of 84 people were
infected with some form of *Trichinella* spp. In 2015, although the cases decreased, more than half of the reported cases were still related to infected bear meat.

The last *Trichinella* study was conducted more than 40 years ago. Studies in other North American mammals establish the presence of *Trichinella* spp. For example, *Trichinella murelli* was found in *Procyon lotor* and *Canis latrans* in 2008, and other species of *Trichinella* are also present in the Americas (Hill et al., 2008). Specifically, you can find *T. spiralis* in the domestic cycle and in the sylvatic cycle *T. nativa, T6, and T. pseudospiralis* (Gamble et al., 2005; Hill et al., 2005; Lindsay et al., 1995; Marucci et al., 2022; Schad et al., 1987). Trichinellosis is still a public health concern even with many food regulations set in place. Muskrat infection with *Trichinella* is very low, below 1%, but the rodent is a candidate for infection (Sterner, 2019).

The last study conducted on the composition of intestinal parasites in Horicon marsh, was in 2013 by Kristina Lapp of Stevens Point (Lapp, 2013). Much of the literature on the intestinal parasites of the muskrat are outdated and are not Horicon marsh specific. Because of this, they do not provide information on the current parasitic biome within Horicon marsh muskrats. Sarah Woody, a graduate student at the University of Wisconsin Oshkosh performed a study on heavy metals and metalloids in sediment, cattail, and muskrat tissue (Woody, 2022). With the help of muskrat trappers, she obtained 36 carcasses from the marsh. The collection of the muskrat was a great opportunity to investigate if *Trichinella* was present in Northeast Wisconsin. If the muskrat was found to be a host for the nematode, then it could support the sylvatic life cycle through its role as prey for predators in the marsh and surrounding area. Surveying the intestines could also
help update information on the species diversity of intestinal helminths of the Wisconsin muskrat.

**Specific aims**

In Wisconsin, game hunting is extremely popular and over the last decades has only continue to increase. For example, between 1986 to 1990 a total of 1,816 permits were given out to individuals looking to hunt black bears in the state (DNR, 2019). In the years between 2014 and 2018, a total of 11,674 permits had been granted. That is an enormous increase in permits in approximately 24 years. Of course, the number of permits would have to increase when 124,053 hopeful applicants applied for the opportunity to hunt black bear (DNR, 2019). Although great business for the state, the possibility for mass outbreaks increases if the proper surveillance and education is not provided or readily available.

My aim through this project was not only to establish the presence of *Trichinella* spp. in Wisconsin, but to map out its distribution as well. With the collection of these data, I hoped to continue the conversation on the importance of public education on the dangers of the nematode. Trichinellosis is a very preventable infection and with continued education the yearly cases can only decrease. Similarly, many people visit Horicon marsh yearly and the muskrat may harbor parasitic agents that can be dangerous to both wildlife and humans. Through this thesis I hope to provide the marsh and the scientific community valuable information on the parasite species diversity so that, if needed, intervention can occur.
Chapter II: Trichinosis

Trichinella survey in WI Wildlife

Introduction

Trichinosis is a disease caused by parasitic nematodes in the genus *Trichinella*. *Trichinella* worms, sometimes called ‘trichina worms’, are commonly found in animals that regularly consume meat either by predation or scavenging. Prevalence of trichinosis is cosmopolitan and infection is common, particularly in wildlife. Humans are rarely diagnosed with trichinosis in developed countries because (a) domestic and agricultural animals, primarily pigs, are usually managed properly and (b) it is uncommon to eat rare or undercooked pork meat. In this chapter, I explore the worldwide economic impact of trichinosis, outline the biology and transmission of the parasite, discuss pathogenesis and control, and finally, report the results of a recent survey for the parasite in Wisconsin *Odantra zibethicus*.

Trichinella economic Importance

The price tag attached to inspecting a pig for Trichinella infection in the European Union was once estimated to be between 18¢ to $3.70 per pig (Pozio, 1998). When one considers the European Union slaughters approximately 190 million pigs annually, it is easy to see that the economic impact of this disease in the food supply is significant. In 1998, the slaughter houses of the 15 EU countries spent a combined total of $570 million to monitor pig carcasses for this zoonotic parasite. The larger the slaughter house the larger the cost to inspect each pig. Many farms opted for the less costly method which would be the pooled digestion method and would run approximately 22¢ in Europe and
somewhere between 25¢ to 70¢ in the United States (Gottstein et al., 2009). The pooled digestion method consists of taking multiple batches of samples and digesting them via artificial gastric juices. The sediment from the digestion is then observed under a microscope for the presence of *Trichinella*. An alternative to the pooled digestion method is the PrioCheck *Trichinella* AAD. This method uses serine proteases to digest tissue samples leaving behind no hazardous material like hydrochloric acid (HCL) all while reducing assay time by approximately a third (Gajadhar et al., 2018). The more costly method, Loop-mediated isothermal amplification (LAMP) would run for approximately $5.90 per test. In recent years, the price per pig has decreased and has become much more affordable. The price per pig for the same pooled method has been lowered to 6 cents, overall costing $1.72 with labor included (Barlow et al., 2021; Kapel, 2005). The ability to run the pooled digestion method or an ELISA in house has lowered the cost per test since samples no longer need to be sent out to be performed by a third party. Even if the slaughter house decided to go the ELISA route, the cost per ELISA was estimated to be 83¢ including personnel. It is much more affordable now than it was back in the late 90s due to the availability of commercially available ELISAs, which detect the present of IgG antibodies.

In Serbia, an economic evaluation was conducted to estimate the cost of carrying out a *Trichinella* surveillance program in order to prevent further health crisis in the country (Milorad et al., 2019). The plan that Milorad et al. set in place allows for a sense of the economic strain the control of this parasite can put on the country and its people. An area with 335, 901 inhabitants was chosen as the study site. Over a ten-year period, the
program was estimated to cost a total of €2,901,682.35 which roughly comes out to
$3,186,910.76. The efforts of the program also include minimizing the presence of rats in
the area since rats were major Trichinella reservoirs in this zone. An additional 17.55%
and 5% of the total cost was allotted to the material and work personnel needed to set up
baits for the rats. Although this initially seems like a huge expense the return on the
investment was going to surpass that of what was initially invested. A total of
€5,101,247.06, or approximately $5,602,687.40, would be saved over the time span of the
project. Fewer infected pigs mean more pigs can go on the market, less trichinoscopy
examinations were needed, and the cost to treat human cases would also decrease.
Overall, this plan would save the municipalities more than they initially spent (Milorad et
al., 2019).

Pozio (1998) suggests that we should not be spending money on every pig that is
slaughtered, and we should take a more strategic approach. Do not test every pig in areas
not epidemiologically important, simply test areas with known outbreaks. A detailed map
can be made by government and state agencies that outline the sylvatic and domestic life
cycles currently active within any country. Not only this but leave the testing exclusively
for animals bred on small farms or wild game. If the European Union would stop testing
pigs that come from industrial farms, where Trichinella spp. is no longer a threat, they
would be able to save $420,000,000 per year (Pozio, 1998).

In the United States, trichinellosis surveillance began in 1947 and within the first 10
years reported 360 cases. In 1983, it is estimated that the economic impact of Trichinella
on the porcine industry was above $400,000,000. This number does not include the
medical costs accrued by those infected with the parasite. An additional $1,500,000 to $2,200,000 was paid in treatment alone (Murrell, 1991). Using data collected from 1978 to 1982 from the CDC, The Carter Center Project, and estimates provided by Canadian agencies; the estimate of cases soars upward of 40,000 individuals during this time period. This cost the public an estimated 86 million dollars in treatment for those affected (Ewen, 1989).

There are studies in the US surveilling outbreaks of *Trichinella* like the one produced by Burke et al. (2008), which outlined the way geographical data can be used to identify potential risk (Burke et al., 2008). Using published data from the last 60 years, as well as information from the *Trichinella* reference center, a map of outbreaks was transposed onto a general blank political map of the Midwest and Eastern US. The location data of all grass-fed swine operations were added to the map, and this allowed for a resource that indicated which farms were near outbreaks and who was in potential danger.

Surveillance studies like these have been coupled with a slight change in procedure for detection during meat harvest. Instead of focusing on the detection at slaughter, detection should be applied during production (Burke, 2008).

In the US, the Trichinae Certification Pilot Program Standards have been put in place in order to ensure that a pig farm is *Trichinella* free. This certification comes with a set of rules that producers must follow in order to certify their livestock as healthy. First, if there is movement from purchase of an animal it must be purchased from another certified source. When the animal is purchased an identification number must accompany the animal and must be recorded. Feed must be purchased from a certified business with
good manufacturing practices set by their respective industry. The feed purchased must be stored in an area that cannot be accessible to other potential hosts like rats. A rodent log must be kept in the vicinity and it must be updated if any rodent is found. The only time wildlife tissue can be fed to any swine when the provider holds the appropriate state permits. If any of the meat is cooked previous to feeding, it cannot be mixed with any other uncooked household meats. A second log that contains cooking and temperature times must be kept at all times. General maintenance of housing facilities must kept regularly, this includes cleaning up of excrement or the carcass of any pig upon death to avoid cannibalism (Pyburn et al., 2005). An isolation period is also recommended for any incoming animals that will join other swine for slaughter.

**Biology and Life Cycle**

Trichinosis can be caused by several species of parasitic nematodes in the genus *Trichinella*. The worms are intracellular and spend their whole life cycle inside the host, which technically acts as both the definitive and the intermediate host due to the parasite’s unique biology.

To understand the biology of this worm, it is important to know that the nematodes hatch from an cyst as a first stage juvenile (J1). Throughout their life they progress through four juvenile stages and then to an adult stage, and each transition is accompanied by a molt of the external cuticle. This allows the worms to grow in size as they age. First stage *Trichinella* juveniles are encysted in skeletal muscle cells of the host where they exhibit anaerobic metabolism. When the muscle is consumed by a predator or scavenger, the infected cells will reach the stomach and the worms will break free due to the HCL
and pepsin mixture within the digestive tract. Once free, the nematodes travel to the small intestine where they molt and copulate approximately two days after being ingested (Gottstein et al., 2009). This molting process into adulthood is completed extremely quickly, especially considering that the worm molts four times. Other nematodes like *Anasikas simplex* completes one molt in 4 to 7 days (Grabda, 1976). The nematode *Rotylenchulus reniformis* takes two weeks to molt from an L2 to L3 (Bird, 1983). An in vitro study on *Brugia malayi* molting suggests that one singular molt from L3 to L4 takes approximately 7 to 8 days (Ramesh et al., 2005). The adult *Trichinella* worms lie in the cytoplasm of the epithelial cells of the intestines where they thread through the serial row of the intestinal cells (Roberts, 2014). Once it has mated, the female will stay in the body between 4 to 16 weeks before being expelled. However, before exiting the host body, the females will birth over 1000 juveniles. The males mature and copulate several times before being swiftly expelled out of the host body (Schmidt & Roberts, 2009). This all happens 30 to 32 hours after the infection has begun and continues over the course of four to six weeks. The new offspring will make their way into the blood stream of the host by going through the lamina propria. The juveniles will invade the circulatory as well as the lymphatic system of the host in order to colonize skeletal muscle. Most of the J1s will be transported out into the body through the hepatoportal system. They are pushed out into the heart, lungs, and arterial system. During the migration stage of the parasite, they can be found all throughout the body in various tissue types. The parasite will travel through capillaries and eventually find muscle tissue where it will reside in the cytoplasm of the cell. Only striated muscle tissue can provide the necessary conditions it
needs to live. If the juvenile finds any other tissue, the cell will die as a result. When the worm reaches skeletal muscle, they begin to change the gene expression of the host’s cell. The larva secretes chemicals that cause the formation of hypertrophic nuclei, causes loss of myofibrils, and increases smooth endoplasmic reticulum resulting in the formation of nurse cells. This nurse cell will then become surrounded by collagen fibers that redirect capillaries which feed the structure and the parasite itself (Despommier, 1998); (Schmidt & Roberts, 2009). The secretions of the parasite will then control the function of the cell which it uses to make the cell re-enter the cell cycle. The cell will permanently be stuck in the G2/M phase of the cell cycle (Jasmer, 1993). This stops the muscle cell from expressing muscle genes that would lead the cell into the proliferative phase (Despommier, 1998; Lee, 2014; Schmidt & Roberts, 2009. The larva stays in these nurse cells until it is eaten by a predator that is a suitable host. Once ingested, the gastric juices dissolve the nurse cell capsules and allow the larva to break out of the cell, also referred to as a cyst, where it can then travel to the intestine to restart the cycle again.

Trichinosis is a zoonotic disease. Due to the proximity of these animals to humans, and because animals like pigs are often consumed by humans, they can become an accidental host to the parasite. In the domestic cycle, pigs are the largest contributor to the fulfillment of the \textit{Trichinella} life cycle. These domesticated animals are indiscriminate eaters, sometimes ingesting waste, which may include offal or trichinous meats. All it takes is one infected individual for a whole pig farm to potentially contract the nematode. Pigs also exhibit cannibalism when they are kept in close crowded quarters. The behavior does not entail eating whole cage mate, but it is common practice for a pig to bite off
other pigs’ tails and ears. This results in the uninfected pig exposing itself to the encysted juveniles which are the infective stage of the nematode. The unknowing human will then kill the pig and ingest the meat. If the meat is undercooked and ingested, the individual will ingest the cysts and spread the parasites throughout their system. Because there are very few predators of humans, humans are considered a dead-end host.

Humans come in contact with *Trichinella* spp. In both the domestic and the sylvatic cycles. Most commonly, this occurs through activities like hunting and consumption of game meat. With dark meat it can be hard to tell when the meat is fully cooked, and the ingestion of active larvae occurs. Even though we are aware that eating raw meat is dangerous, some people around the world intentionally eat meat raw. In many regions of the world it is culturally appropriate or a delicacy to eat raw meats. Coincidentally, the incidence of trichinosis in these regions is much higher than in countries where eating raw meat is not common practice.

**Morphology**

An adult male worm is between 1.4mm to 1.6mm in length. It is slender near the anterior side with the anus nearly terminal and has a large copulatory pseudobursa. The adult female is usually twice as long as the male, and tappers anteriorly with the anus being nearly terminal. The vulva of the females is near the middle of the esophagus, and the esophagus is about 1/3 the length of its body. It has a single uterus that is filled with developing eggs on the posterior side (Roberts et al., 2009). Although there are no key morphological features that set the nine characterized *Trichinella* species apart, the males and females can easily be distinguished from each other based on size. Molecular
analysis like allozyme and or ribosomal DNA analysis, must be used to identify different species (Lee, 2014). Multiplex PCR can differentiate between all genotypes of *Trichinella*. The ITS1, ITS2, and V segments of ribosomal DNA can be amplified for identification (Zarlenga et al., 1999). The amplicon can then be taken and visualized with the use of gel electrophoresis. It is only through these molecular analyses one is able to differentiate an adult *T. spiralis* from any of the other nine identified species of *Trichinella* (Lee, 2014).

**Figure 1.** Male (top) and female (bottom) *Trichinella spiralis* adults recovered from white rats four days post infection (Gould et al., 1957).

**Transmission**

Anthropophilic animals, like rats and pigs, tend to display cannibalistic behaviors. *Trichinella spiralis* relies this behavior to move from host to host. The scenario is as follows, the rat will feast on another rat and subsequently ingest the juvenile larvae within the muscle. Once situated in striated muscle, *Trichinella* spp. incapacitates rodents
by restricting movement. The lack of motility makes it easier for them to be caught and eaten by other animals, in this case pigs (Lee, 2014). The pig, whether on a farm or an industrial slaughter house, will be slaughtered for consumption. It is up to every individual to properly cook the meat they eat. Noeckler et al. (2019), describes the temperature at which pork should be cooked at in order to inactivate the encysted Trichinella larvae. Although humans are dead end hosts, they exhibit behaviors that feed back into the cycle, such as throwing out trichinous offal for wild animals and domestic animals to eat.

In the sylvatic cycle, transmission is reliant on cannibalism and on hunting and scavenging behavior. Many of the infected animals are those that exhibit hunting and scavenging as a preferred way to obtain a meal. In arid zone, animals like hyenas (Hyaena hyaena), cheetahs (Acinonyx jubatus), and leopards (Pantera pardus) harbor the most infection. In temperate areas there are foxes (Vulpes vulpes), raccoons (Procyon lotor), and coyotes (Canis latrans) and in frigid zones grizzlies (Ursus arctos horribilis), polar bears (Ursus maritimus), brown bears (Ursus arctos), and fox (Vulpes lagopus) are the most affected (Pozio, 2000). All of these animals are scavengers or hunters. They will continue to take potentially infected meals for the rest of their lives while simultaneously assisting in the transmission of the nematode.

**Pathogenesis and diagnosis**

Most of the fear that comes with eating pork has faded with introduction of testing for the parasite. A portion of porcine muscle was excised and placed between two slides. The sample was then observed under a microscope. The presence of cysts would alert to the
contamination of the pork, and could therefore be removed to avoid further spread of the infection. Although this method was very effective, the act of taking individual slices of pork and visually inspecting one sample at a time was extremely time consuming. A new pooled method was introduced in 1967 (Gamble et al., 1999). The meat from multiple pigs were digested at once with the use of artificial digestive juices. This method although helpful, also comes with some downfalls. If the meat is tested and comes back negative then it would indicate the pork was clear of infection and safe to eat. However, if the test comes back positive the meat will be tested again in smaller groups in an attempt to locate the infected carcasses. The importance of controlling the parasite has not decreased to this day. In Europe, meat from slaughter house sold for human consumption must be tested especially if the animals is at high risk for contracting an infection. In addition, pig pens must be kept clean and rodents must be kept under control. If any pigs are to be fattened for slaughter they are not to be let outside after four weeks post initiating the process.

Infection has two different stages, the enteral phase and the parenteral phase. The enteral phase describes the process of the larvae hatching from the cyst, and molting into adults. For many, this phase can be asymptomatic. For those that do have symptoms they can often be confused with other enteral disorders like food poisoning or indigestion. The clinical signs include mild diarrhea and nausea. It is only when an individual is infected with moderate to severe worm loads that other more alarming clinical signs present themselves. The symptoms include abdominal pain, vomiting, malaise, and low-grade fever (Capo & Despommier, 1996). Even at this point the unsuspecting victim will most
likely go undiagnosed or misdiagnosed due to how common these symptoms are to other non-threatening illness. Most patients are going to be diagnosed during the parenteral phase, which involves the migration of the larvae outward into the body. An individual can start to experience periorbital and facial edema, difficulty swallowing, and in some cases a paralysis-like state (Capo & Despommier, 1996). A variety of other symptoms can present themselves like fever, headache, and rashes. In extreme cases, an infected individual can be diagnosed with neurotrichinellosis. The migratory larvae find themselves lodged in the brain and the trichinae encyst within the brain, wreaking havoc on the host’s central and peripheral nervous system (Bruschi et al., 2013; Capo & Despommier, 1996). It cannot be emphasized enough that the migration process of the nematode is the most dangerous part of the infection process. In extreme clinical cases, the heart cannot withstand the burrowing of the larvae out of the heart. This leads to inflammation of all valves (endocarditis), inflammation of the heart walls, specifically the middle layer (myocarditis), and can ultimately lead to heart failure.

Wild animals that have been naturally infected with *Trichinella* spp. do not show any typical signs of infection. Unlike humans that show a variety of clinical signs that would suggest infection; wild animals like lizards, birds, and ruminants will resume their daily activity unbothered (Mukaratirwa et al., 2013). In a study conducted with laboratory mice, following infection with *Trichinella spiralis* the activity level of infected mice was significantly decreased. Compared to the uninfected control mice who maintained relatively average motility (Zohar & Rau, 1986). A second study on social interactions between mice demonstrated that when mice have a high number of larvae in muscle...
tissue, they were less likely to be social. These mice also showed a decrease in their exploratory behavior when compared to their uninfected counterpart (Edwards, 1988). Although wild animals might not show physical symptoms like humans, perhaps this behavioral change in laboratory rats demonstrates the nematode’s way of increasing its chances of transmission into a new host.

In humans, the best way to diagnose for a Trichinella spp infection is through a muscle biopsy. A portion of skeletal muscle is taken from the individual, flattened between two slides, and observed under a microscope. The technician must be careful as a newer infection might appear as healthy tissue. Microscopy can be used in human and animal identification. Upon inspection, one can see the free-floating worms in the striated muscle tissue not yet encapsulated. The best practice is to stain the tissue so that one can observe the changes in striation patterns caused by the presence of the newly arrived worm (Capo & Despommier, 1996). When using microscopy as a diagnostic test, it is best to follow it up with a second test know as an ELISA. An ELISA for IgG antibodies would show positive for most patients by day 14 when clinical symptoms push the individual to seek medical help (Capo & Despommier, 1996). By day 50, the ELISA test will reach a sensitivity of 100%. A third diagnostic test, DNA amplification, is a perfect choice for detection of ITS regions and the V segment; although it is not currently available commercially.

The European Center for Disease and control has something called a case definition. The patient must meet one requirement from each criterion group in order to be diagnosed with a positive case of trichinellosis. From a clinical aspect the patient must have: fever,
muscle soreness, gastrointestinal symptoms, eosinophilia, or other clinical symptoms. *Trichinella* larvae must be present in one of the diagnostic test previously described above (Biopsy, ELISA) or also suggested, Western blot (Gottstein et al., 2009). The western blot is used to detect secretory/excretory proteins secreted by the cuticle of the worm (Gamble et al., 2004). Lastly, the individual must have eaten infected meat confirmed through laboratory testing, suspected to be infected while being tested in a laboratory, or directly linked to a person with a confirmed infection. As for non-human diagnostic methods, the diagnostic method is only used on an animal upon being slaughtered for sale. The tests are simply used to note the present or absence of the parasite and is directly tied to control of *Trichinella* spp.

**Prevalence**

Prevalence refers to the proportion of cases of a disease at any time. Keeping record of prevalence for this parasite is relatively hard to do when talking about the sylvatic life cycle. The only time wild animals are tested for *Trichinella* are upon being butchered for meat or if a study is specifically targeting the parasite. Globally, we do have records of pig prevalence because a great quantity of our global swine population is tested upon being slaughtered. A meta-analysis by Eslahi et al. (2022), referencing 60 studies across 32 countries reveals the global prevalence of *Trichinella* in pigs. A combined sample size of 751,167,472 pigs reported in 60 manuscripts were examined in this study. Out of the that sample size the pooled prevalence was 2.0%. The prevalence in countries like Poland has stayed consistently low since the 1950s. In recent years, the prevalence of the nematode has actually decreased to a much lower percentage (0.000088%) (Bilska-Zajac...
et al., 2021). Similarly in China, many provinces reported prevalence of *Trichinella* spp. at around 5% to 7% even by the end of the 90s (Takahashi et al., 2000). In Northeastern United States, data collected from six states showed the prevalence from 0% to 0.69% in domestic pigs (Gamble et al., 1999). The infection rates in the United States appear to be well below the 2.0% range previously presented.

In the arctic and subarctic, the presence of *T.nativa* is of concern. The prevalence of the trematode has been reported at as high as 60%. The major contributors to this percentage being *Canis lupus*, *Ursus arctos*, and *Ursus maritimus*. *Ursus arctos horriblis* within this same region had an outstanding 47% prevalence rate. *Ursus americanus* was not too far behind with a 27.5% prevalence rate. In Greenland, *Vulpus lagopus* populations showed to have high prevalence of infection and in Finland, *Felis Lynx* (40.5% and between 7.7% to 67% respectively). In Estonia, *Canis lupus* infection rate is an alarming 75% prevalence. The wild boar in this area are the least affected at 0.7%, it appears to not be a great candidate for *T.nativa* (Pozio, 1998, 2000).

**Control**

Human infection with *Trichinella* spp. was much more common during the 19th and 20th century than it is currently. In fact, autopsies performed during this time showed that approximately 20% of Europeans had been infected with the roundworm (Gamble et al., 1999). In recent decades, eastern Europe still experiences trichinosis because *Trichinella* can be found in several animal populations. Those animals include horses, pigs, and wild boar (Kapel, 2005). Much of the trichinous meat comes from family-owned farms and from wild game. Family farms which only sell or trade meat locally are not required by
law to test the meat before being placed on the market for sale. This lack of caution coupled with illegal meat trade has led to outbreaks in several European countries (Kapel, 2005). A second problem presents itself due to improper practice on the part of game hunters. Carcasses are often left on hunting grounds and carnivores like foxes, and raccoon dogs take gratuitous meals from the abandoned carcasses. This practice allows three species of sylvatic *Trichinella* (*T. britovi, T. nativa, T. pseudospiralis*) to thrive in places like Italy, Germany, and Spain. A fourth species, *Trichinella spiralis*, also continues to be spread due to human malpractice. The improper disposal of slaughter house offal allows for wildlife to ingest the contaminated tissue furthering the problem.

The consumption of exotic meats has introduced additional *Trichinella* species into a whole new range. The poaching of polar bears and marine seals for human consumption introduces *Trichinella nativa*. The illegal trade of crocodile meat from Zimbabwe introduces *Trichinella zibabwensis* and *Trichinella papuae*. Currently, the geographical distribution of *T. zibabwensis* and *T. papuae* is limited to Ethiopia, but these two species have the ability to infect a variety animals since it is not limited to cold blooded animals (Kapel, 2005); (Barker et. al, 2006).

To keep not only human infection low, but to lessen the sylvatic cycles, public education must be continued on a global scale. A prominent global program must be set in place to educate the public on the dangers of consuming raw or undercooked meat. This campaign must include information on wild animals that can be carriers for the nematode so that the public understands that this is not limited to pigs. Additionally, it is important to use veterinary care. Having a trained licensed professional regularly inspecting animals can
be a detrimental step to preventing the sale of trichinous meat. Lastly, even if all of the above steps have been taken, it is still important to use the artificial digestion method to test for the present of any species of *Trichinella*. The digestion method is suggested for slaughter house meats and for personal game meat. As previously mentioned, the problem does not lie with big industrial slaughter house as much as it does with family-owned farms. If an individual is purchasing from a family-owned farm that cannot afford to test or simply does not test, there are steps one can take to protect themselves. The first and most important is making sure that the meat has been cooked all the way through. The meat must not contain any pink and the muscle fibers must have the ability to be torn apart. Cooking is the simplest and best way to prevent any infection. A second option would be to freeze the meat. This method is only recommended for pork potentially infected with *T. spiralis* as there are freeze resistant species of *Trichinella*. *Trichinella britovi* and *T. native* are two species that can survive months to years in frozen muscle tissue and still be viable (Pozio, 2023). Lastly, irradiation can be performed on sealed foods to inactivate the parasite. This method can only be performed in some countries as not everywhere in the world permits irradiation for food items. Contrary to popular belief, curing meats with commercial salts or smoking meats will not inactivate the parasite. This is true for both commercial or game meats, and both meats should only be cured or smoked after the meat has gone through testing processes rendering it safe to consume (Gottstein et al., 2009). Mauritz Sterner proposed that a new *Trichinella* survey be conducted in the state of Wisconsin (Sterner, 2019). The last survey of the black bear in Wisconsin was published in 1977; that is 46 years without data on the prevalence of
the nematode in black bears. Another concern is the freeze resistance of the worms, as
also mentioned by Sterner (Sterner. 2019). Some species of Trichinella have been
reported to survive freezing for up to 28 months and still be viable. It is thought that the
older the infection, the thicker the nurse cell wall, and thus the more resistant to freezing
the worm will be. The best known species to be resistant to freezing is T. nativa.

Trichinella native has been observed to survive for years in fox tissue in temperatures as
low as -18°C (Lacour et al., 2013). There is some variation even within the same species
depending on location. Trichinella nativa found in the high arctic latitudes of Alaska
showed more resistance than isolates found in Norway. Additionally, within the
Norwegian isolate, location mattered. The isolates gathered inland where more tolerant to
freezing when compared to the coastal isolate (Davidson et al., 2008; Lacour et al.,
2013). What did remain true was that all three isolates were extremely resistant to
freezing and thawing cycles. A genotype called Trichinella T6 has a similar distribution
as T. nativa. It is present in Artic and sub-arctic zones of North America and also
displayed freeze tolerance to -18°C. Other species like T. spiralis, T. britovi, and T.
pseudospiralis were also seen to display some form of freeze tolerance as these three
were able to survive up to eight weeks in -18°C (Lacour et al., 2013). Trichinella spiralis,
T. nativa, T. britovi, 3 strains of pseudospiralis, T. murrelli, T6, and T. nelsoni were
experimentally tested for freeze tolerance (Malakauskas & Kapel, 2003). Each group was
frozen at -18°C, -5°C, and 5°C for 1 to 40 weeks. T. spiralis and T. pseudospiralis
survived -18°C for 1 week., T. britovi, T. murrelli, T6, and T. nelson did not make it to the
one-week mark. The only isolate to make it past 1 week was T. nativa, this isolate
survived almost 4 weeks. In the -5°C, all isolate did well. The surprise here was that the non-encapsulated isolates were able to make it to 4 weeks, this suggests that they do possess a small amount of resistant to cooler temperatures. Lastly, in the 5°C category, reproductive ability was observed to continue unaffected in this temperature (Malakauskas & Kapel, 2003).

The were many anthelmintics discovered before the 2000s, such as benzimidazoles, sulphonamide and organophosphates. Many of these types of drugs are still being used in veterinary care today. Some of these drugs are safer than others. For example, benzimidazoles and the imidazothiazole, levamisole. Both fairly safe to use, although they can come with some side effects that are rarely seen like embryotoxicity, muscle tremors, and defecation. Other drug classes like organophosphates are generally more toxic and require increased care when dosing an animal. On the other hand, macrocyclic lactones are generally safe for mammals and can often times be administered at 10 times the recommended dose without adverse effects (Vercruysse & Claerebout, 2014). The discovery of ivermectin came from the collection of a soil sample. Dr. Satoshi Omura collected the soil sample near a golf course in Honshu, Japan. He isolated a gram-positive bacterium that would be handed over to Dr. William Campbell at Merck laboratory. The result of this collaboration was the production of a family of macrocyclic lactones of which ivermectin was the most effective against helminths. This wonder drug has effects on not only ectoparasites, but endoparasites as well and was ultimately used to control infection. The multifaceted drug could regulate cholesterol in diabetics, control cell growth of multiple cancers, and even reduce survival of many arthropod vectors (Rahman
et al., 2017). It was due to its many abilities that drug was immediately placed on the market to treat both human and non-humans alike. In the case of *Trichinella*, the effects of ivermectin have been observed and the results have been favorable. Experimentally infected mice were treated with ivermectin at different days post infection (Soliman et al., 2011). The mice were treated at 0, 5, 15, and 35 days after infection and the mice were then necropsied to observe the effect of the drug on the worms. The mice that most benefitted from treatment were those treated on day zero. These mice had zero to three worms recovered. As the days passed and the larvae were allowed to enter the host muscle tissue the efficacy of the drug decreased. To those mice administered ivermectin on day 35 half of the worm burden was found when compared to control mice, still effective but the effects were lowered. A second study provided the drug on days 4, 10, and 35 (Soliman et al., 2011). The pattern was similar; the sooner you can treat the infected individual with the ivermectin the lower their worm load. When serology was performed the levels of AST (aspartate transaminase), ALT (alanince transaminase), urea, and creatine were highly elevated in untreated mice. The mice that received the treatment on day 4 and 10 showed significantly decreased levels of the aforementioned (Soliman et al., 2011).

As an anthelmintic, ivermectin can be used to treat many farm animals including horses, pigs, sheep, and goats. The primary purpose of its use is to treat gastrointestinal roundworms, but it can be used to treat ectoparasites like lice, mites, and often times arthropods. Ivermectin has even been used to treat river blindness and lymphatic filariasis (Smith, 2021). The use of this macrocyclic lactone in conjunction with good farming
practices like controlling rodent populations, providing feed from licensed sources, and avoiding bad hygiene will repel unwanted synanthropic organisms. In addition, keeping logs, quarantining incoming animals (purchased from a licensed vendor), and keeping well-built enclosures will keep cattle as well as other farm dwelling animals *Trichinella* free.

**Materials and Methods**

In Spring of 2021, Sarah Woody collected 36 muskrats between March 2nd and 14th; with the help of trappers from Horicon Marsh, Wisconsin. The muskrats were retrieved from body grip animal traps lodged within the ice and geolocation data were taken at the time the muskrats were retrieved. Four different zones of the main pool were sampled. Those zones are named Main Pool North, Main Pool South, Teal, and Radke. From Main Pool North and South, nine muskrats from each zone were sampled. In Radke 13 muskrats were retrieved and from Teal a total of 18. The muskrats were initially taken by the hunters so that they could dry the muskrat, skin them, and return the carcass to Sarah for dissection at a later date. In May of 2021, carcasses were unfrozen nine per day and dissected. For the purpose of this study, tongues were retrieved from every muskrat. Every tongue was individually placed in its own plastic zip lock bag, labeled, and placed in a -20°C freezer to be processed at a later time. In January of 2023, nine more muskrats were collected at Horicon Marsh and their tongues harvested. When ready to be digested, the tongue was set out in room temperature to thaw. A perimeter was set up to keep any spills contained. Within the perimeter worm hooks, surgical scissors, a waring blender, two digital heating plates with magnetic stir bar, two thermometers, and an ice bucket
were placed. Milli-Q water was collected from the UWO water purifying system. The Milli-Q water was placed in a sterile 1000mL beaker in order to make artificial digestive fluids. The artificial digestive fluids were made using 1% pepsin (1:10,000 IU) and 25% hydrochloric acid. For the digestive fluid to go above the rotating blades, 50mL of digestion fluids were used in this experiment.

In order to make the artificial digestion fluids, a protocol provided by Dr. Mason Reichard was adapted to meet the needs of muskrat tongue digestion (Reichard et al., 2008). Twenty five percent HCl (16 ± 0.5 mL) was added to 2 L of Milli-Q water preheated at 46 – 48 °C in a 3 L glass beaker. It was kept at this temperature because too low of a temperature results in incomplete digestion; too high of a temperature deactivates the enzymatic properties of pepsin. A stirring rod was placed in the beaker and the solution was maintained at the same temperature (46 - 48 °C). Once up to temperature, I added 10 ± 0.2 g of pepsin (1:10,000 NF, 1:12,500 BP, 2,000 FIP) to the acidic solution. For laboratory safety and to maintain the pepsin active, the sequence of adding digestion fluid components must be adhered to. Once the pepsin dissolved in the acidic solution the fluids were ready to be utilized.

Before digesting the tongue, the superficial layer that cannot be digested was removed using surgical scissors. The superficial layer was frozen for at least 24 to 48 hours, and then place in biohazard trash to be autoclaved. The remaining tongue tissue was then cut into 0.5cm strips, which helped with the blending process. The strips of the tongue were introduced into the bottom of a Waring blender, and 50mL of digestive fluids were
poured into the cup. The blender only came with two speeds, low and high. Each tongue was blended for 1 minute, 30 seconds in low speed and 30 seconds on high speed. This was enough for the tongue tissue to be broken into smaller pieces that would be digested in the allotted time frame. A second heating plate set to 37°C was prepared simultaneously. The tongue smoothie was transferred to the 500mL glass beaker and placed on a heating plate set to 37°C. A stirring rode was placed inside and was set to spin at 700rpm. An additional 50mL of digestive fluid was used to wash the inside of the blender and the fluid was poured into the beaker with its corresponding sample. The total volume used to aid in the digestion of every tongue was 100mL. The blended tissue was allowed to spin for approximately 30 minutes at 37°C. After 30 minutes, the mixture was immediately set to cool on ice for 20 minutes. After 20 minutes, all sediment had settled at the bottom of the beaker. The sediment was decanted between three and five times with Milli-Q, depending on the amount of cellular debris. Every wash and all sediment recovered was observed under a stereomicroscope at 40X magnification. If any larvae were recovered, the worms were washed in saline solution and placed in vials containing 80% ethanol.

Results

Out of the 45 muskrat tongues sampled, no muskrats displayed signs of any *Trichinella* spp. infection or were thought to be infected. The prevalence within this group of muskrats was 0%.
<table>
<thead>
<tr>
<th>Location</th>
<th>Number of Muskrats sampled</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main Pool North</td>
<td>9</td>
<td>0%</td>
</tr>
<tr>
<td>Main Pool South</td>
<td>9</td>
<td>0%</td>
</tr>
<tr>
<td>Radke</td>
<td>13</td>
<td>0%</td>
</tr>
<tr>
<td>Teal</td>
<td>14</td>
<td>0%</td>
</tr>
</tbody>
</table>

Table 1 Number of muskrats sampled, location, and prevalence percent

**Discussion**

The possibility of finding *Trichinella* in muskrats was very low. The muskrat is a compatible host for the nematode, but it appears to be rarely infected. In a study conducted by Rausch et al. (1956) on the occurrence of *Trichinella* larvae in Alaskan furbearers, only one muskrat was infected out of 11. A study conducted by Beckett and Gallicchio (1967) showed very similar results. Out of 130 muskrats recovered from Portage County, Ohio between 1963 and 1964 only one muskrat had the parasite. A singular encysted larva was found in the diaphragm of the muskrat. Although perhaps not as dangerous for consumption as other organisms, the muskrat still needs to be surveilled on a regular basis. There are people who rely on the muskrat for sustenance. For example, in Alaska the muskrat is significant because it is incorporated into human diet during certain seasons of the year (Robert Rausch et al., 1956). In the state of Pennsylvania, the muskrat is not only eaten by humans, but by swine on farms (Schad et al., 1984). As previously discussed, pigs are part of the domestic cycle and introducing *Trichinella* to such a competent host could lead to a catastrophe for livestock or for any unlucky consumers. The trappers of Horicon Marsh collect these rodents year after year. I believe
it would be prudent to continue to test the carcasses of any muskrat caught. These muskrats are prey to coyotes, foxes, and many other predators that call the marsh home. They could be playing a role in the sylvatic cycle of *Trichinella* spp.
Chapter III: Helminths

Intestinal Helminths of Wisconsin Muskrats (*Odontra zibethicus*)

Common Intestinal Parasites

Muskrats serve as a reservoir for many pathogens including hemorrhagic disease, leptospirosis, ringworm, and pseudotuberculosis. In addition, they can also be carriers of many types of parasites like tapeworms, roundworms, and flukes whose effects on the muskrats we know very little of.

Fecal Oral Transmission

*Giardia* and *Cryptosporidium* are zoonotic protozoan pathogens often transmitted by mammals via waterborne infections (Bitto & Aldras, 2009; Erlandsen, 1990).

Transmission is fecal-oral, and is associated with fecal pollution in water sources. They are able to thrive in many types of settings since the cyst stages of these parasites have high environmental stability and low infectious dose levels. Not only do they have high environmental stability but they are also resistant to several types of water treatment methods like chlorination (Bitto & Aldras, 2009; Erlandsen, 1990). *Giardia* trophozoites can usually be found attached to the microvillus of the small intestine, specifically in the duodenum and the jejunum. The life cycle of *Giardia* involves trophozoites that form cysts within the small intestine of an animal which will eventually be passed in the feces. The cysts will then go out into the environment where they can stay infective for a minimum of two months when in cold temperatures (Erlandsen, 1990). When one has giardiasis the individual will experience abdominal discomfort, nausea, and diarrhea that can last between seven and 10 days. It is possible that the individual will not feel any
symptoms since most infections are asymptomatic. Not only does giardiasis infect humans but it also affects several other animals including deer, dogs, cats, raccoons, and muskrats. Much like *Giardia*, *Cryptosporidium* can be asymptomatic in some individuals, but causes acute diarrheal disease in others. Abdominal discomfort and nausea are some of the same symptoms both of these parasites have in common. Trophozoites live in the microvilli covered surface of the epithelial tissue of the ileum and colon. They then mature to the oocyst stage that is shed in the feces. In some cases of delayed gut motility, the parasite will excyst in the host intestine and reinfect the host leading to a continuous cycle of reinfection (Erlandsen, 1990).

In a study conducted by Bitto and Aldras (2009), 44 muskrats were collected from northern New Jersey and Pennsylvania. Of those 44 muskrats, 22 of them were collected from Stockholm, New Jersey and 22 were collected from Ackermanville, Pennsylvania (Bitto & Aldras, 2009). Out of the 44 specimens, 19 of the 22 muskrats from New Jersey, tested positive for *Giardia*. The remaining three did not have an infection, and eight of the muskrats tested positive for coinfection as they were also parasitized by *Cryptosporidium* (Bitto & Aldras, 2009). So, in total 86.3% of the samples from New Jersey tested positive for a *Giardia* infection, 36.3% had a coinfection, and 13.6% were parasite free. Of the rodents from Pennsylvania 10 of the 22 (45.5%) tested positive for *Giardia*, and five of the 22 presented coinfection with *Cryptosporidium* (Bitto & Aldras, 2009). This particular study shows that the infection of muskrats with both or either of these two parasites, *Giardia* and *Cryptosporidium*, is considerable. The parasite was able to be found in four different regions within the states of New Jersey and Pennsylvania.
A second study looked at infection rates in the states of New Hampshire, New York, Vermont, and Maine. All of these states, with the exception of Maine, had previously experienced outbreaks of waterborne illness. The outbreaks were mainly giardiasis outbreaks. In this same study, Minnesota was included and later Massachusetts as they experienced a giardiasis outbreak during this time period (Erlandsen, 1990). Two different methods were used to determine if the muskrats were infected. If the animal was kill trapped, then cysts were used to determine the presence of the parasite within the rodent. The cysts were obtained via fecal float for examination. If the animal was live trapped and was able to be necropsied within an appropriate amount of time, then the scrapings from the muskrat intestines were used to establish the presence of the trophozoites. The time frame for this to happen and give appropriate results would be approximately 15 hours from the time of host death (Erlandsen, 1990). Seven hundred ninety fecal samples were collected and 219 intestinal scrapes were collected for a total of 1009 samples total. The results between the two types of samples examined showed very different results. The fecal samples showed a much lower infection rate than the samples taken from the intestines of the rodents. The prevalence of *Giardia* infection was 36.6% when examining the fecal samples. When examining the intestinal smear, those samples showed a much higher prevalence of 95.9%. The same was observed with beaver samples, when looking at fecal samples only about 80% of the trophozoite positive beavers were identified. In muskrats, only 61% of trophozoite positive rodents were able to be identified via fecal examination (Erlandsen, 1990).

**Indirect Transmission**
Macroscopic parasites are also prevalent in muskrats. These include digenean trematodes, cestodes, and nematodes, which as a group are referred to as “helminths”. In a study of 50 Illinois muskrats, 60% were infected with at least one helminth parasite. The most common helminth found was the digenean trematode *Echinostoma trivolvis*, which was in 40% of the muskrats (Zabiega, 1996). This parasite causes ulceration and bleeding of the intestinal mucosa and is thought to be the most common trematode of all warm-blooded semiaquatic vertebrates (Zabiega, 1996). The next parasite described in this study was the digenean trematode *Quinqueserialis quinqueserialis*. Nine of the 50 muskrats were infected with this parasite. *Quinqueserialis quinqueserialis* can be found everywhere in the United States and has the same distribution as that of the muskrat (Zabiega, 1996). While it is a common parasite, the intensity of infection is generally very low.

A study conducted in Maine collected 104 muskrats for the examination of the presence of helminths, 78% of which were infected with *Q. quinqueserialis* (Meyer & Reilly, 1950). The cercaria of the digenean like to encyst in snails and on vegetation which the muskrats like to eat. Another digenean trematode, *Notocotylus filamentus*, was located in the large intestine of 36 of the samples collected. The life cycle of this specific species is not known. However, given the life cycle of other *Notocotylus spp.*, the infection comes after ingestion of infected snails (Fried & Gainsburg, 1980; Meyer & Reilly, 1950). A small percentage of these animals were also infected with the digenean trematode *Nudacotyle novicia*. The cercaria stage of this parasite encysts on the vegetation that the rodent eats. Other digenean trematodes found include *Pseudodiscus zibethicus*, a
digenean trematode that encysts on vegetation, and was present in 45% of the muskrats (Meyer & Reilly, 1950). *Echinoparyphium recurvatum* is a digenean trematode that was found in the small intestine, and was present in 18% of the samples. The cercariae of this parasite likes to encyst in snails. *Echinostoma revolutum* was found in the small intestine and infected 22 of the muskrats. The infection with this parasite was most likely contracted from eating infected gastropods or finger nail clams. *Plagiorchis proximus* is another that was collected from the small intestine and was present in six of the samples. The cercariae of this parasite like to encyst in *Chironomidae* spp., *Odonata* spp., *Culicidae* spp. larvae, and *Ephemeroptera* naiads (Meyer & Reilly, 1950). Lastly, the final parasite found in this study was the intestinal tapeworm *Hymenolepis evagina*, which was found in 43 samples. The life cycle of *H. evagina* was not known when this paper was published (Meyer & Reilly, 1950) and no further information is available on the life cycle of the parasite. In the study of the 104 muskrats collected, 91 of them were infected with some sort of helminth. Only three total muskrats where endoparasite free, one had no parasite at all and the two others only suffered from mites. The non-infected muskrats were very young and it is believed to be the reason why they were not infected.

A review of the most common intestinal trematodes conducted by Ganoe et al. (2020) delineates the previously discussed parasites with the addition of the digenean trematode *Wardius zibethicus*. The most commonly reported cestodes are *Hymenolepis* spp and *H. taeniaeformis* (Ganoe et al., 2020). Although cestodes can be found in muskrats they do not have as high prevalence as the trematodes. In all of the publications reviewed for Ganoe et al’s (2020) article none of the studies found a prevalence above that of 59%.
This was so for *Hymenolepis* spp which had an average prevalence of 0 to 59%, and the higher end of infection was mostly seen in the northern most parts of the United States (Ganoe et al., 2020). The last type of intestinal parasite described by Ganoe et al. (2020) was in the Phylum *Acanthocephala*. These parasites are known as the spiny-headed worms. Some common species reportedly found were *Corynosoma* spp. and *Polymorphus* spp. The prevalence of these two were fairly low and the intensity of infection ranged from one to 40 worms per host (Ganoe et al., 2020).

For comparison, in Southern Texas an experiment on 36 muskrats caught with the help of the Game, Fish, and Oyster Commission. A total of 16 muskrats were examined and three types of parasites were found: the digenean trematode *Echinochasmus schwartzi* and two nematodes *Rictularia ondatrae* and *Litomosoides carinii* (Chandler, 1941). In Chambers County, 6 types of parasites were found. The trematodes *Echinochasmus schwartzi*, *Phagicola lageniformis*, and *Nudactyle novicia* were present (Chandler, 1941). The nematodes *Strongyloides ratti*, *Longistriata dalrymplei*, and *Rictularia ondatrae* were also found within the samples collected, but no cestodes were collected in this study (Chandler, 1941). The prevalence of the parasites within the samples are all below 50% with the exception of *E. schwartzi*, which seemed to be the most common parasite found. The parasite population in Texas was different from those found in the northern parts of the United States.

Barker (1914) detailed the commonly found parasites in the American muskrat populations and where in the intestines they can be found. *Echinostomum coalitum* is a trematode with an elongated and mostly flat body that can be found in the duodenum of
the muskrat intestine. *Echinoparyphium contiguum* has a spindle or boat shaped flattened body and can also be found in the duodenum. *Echinostomum callawayensis* has a body with rounded ends which is referred to as spatulate and can be found in the duodenum. *Echinostomum armigerum* will have an elliptical flattened body, and much like the other echinostoma can be found in the duodenum. Also found in the duodenum: *Catatropis fimbriata, Hemistomum craterum* (also cecum), *Plagiorchis Proximus, Hymenolepis evaginata, Anomotaenia telescopica* (Barker, 1914). In the cecum, *Notocotyle quinquiseriale* and *Wardius zibethicus* may be present (Barker, 1914). *Trichuris opaca* which has a cylindrical body can be found in the duodenum of the host. *Trichostrongylus fiberius* who has a thread-like body can be found in the duodenum and cecum of the host. *Capillaria ransomia* which was described as having a capillary body can be found in the duodenum as well.

Lastly, in her thesis work, Lapp (2013) describes some of the commonly found trematodes within Wisconsin muskrats. For her study she collected 49 muskrat carcasses with the help of trappers from three sites in Wisconsin. The three sites were in Dunn County, Horicon Marsh, and Waupun. There were three different species of parasites, were found in Dunn County *Echinostoma* spp., *Quinqueserialis quinqueserialis*, and *Notocotylus urbanensis*. *Quinqueserialis quinqueserialis, Taenia taeniaeformis, Echinostoma* sp, and *Hymenolepis* spp. were recovered from 15 carcasses collected in Horicon Marsh. In the Waupun site, *Echinostoma* sp, *Quinqueserialis quinqueserialis*, and *Notocotylus urbanensis* were recovered from the samples. In Dunn County, approximately 72% of the samples were infected with at least one type of parasite,
Horicon Marsh had approximately 73% of the samples infected, and the Waupun site had approximately 58% of the samples infected. Lapp’s (2013) findings coincided with much of the other literature available during the time of her work. The cestode *T. taeniaeformis* did not follow the trend described in other studies. This particular parasite was found on a cyst that was located on a lobe of one of the muskrat’s liver. Lapp’s data showed no significant difference in parasite intensity between any of the three locations. What was significantly different was the mean parasite intensity between adults and juveniles in regards to *Echinostoma* spp. In other rodents the younger they are, the least likely they are to be parasitized by any type of endoparasite (Winternitz & Yabsley, 2012).

Lapp (2013) goes on to mention other parasites commonly found in muskrats but not in her study as: *Alaria* *mustelae*, *Allossogonoporus* *marginalis*, *E. revolutum*, *Fibricola cratera*, *Opisthochis tonkae*, *Ptyalincola ondatrae*, and *Schostosomatium douthitti*. As for the cestodes, *Anomotaenia telescopia* is a tapeworm that infects insects and through ingestion makes the muskrat the incidental definitive host. Nematodes infecting the rodents would include *Ascaris* *sp*, *Capillaria hepatica*, *C. michiganesis*, *C. ransomia*, and *Trichostrongylus fiberius* (Lapp, 2013).

The list of parasites that can be found in the intestines of these rodents is quite large. This excludes parasites that are found in other sites such as blood, liver, reproductive tract, and other organs. It is surprising that so little information is present on an animal that is of such high value monetarily and of importance to public health. There also seems to be some contradiction between available information on habitat favorability. The article by Lapp (2013) however, did not find a significant difference between habitat location and
the type of parasite infecting the rodents within Wisconsin. Investigating what pathogens are present can be beneficial because the rodent is a reservoir, accidental, and intermediate host for many parasites which can be a danger to human health. A complete list of muskrat parasites identified in the literature is included as an addendum to this chapter.

**Materials and Methods**

Forty-five muskrats were retrieved from body grip animal traps, and geolocation data were taken for 36 rodents by Sarah Woody and nine by myself. The muskrats were initially taken by the hunters so that they could dry the muskrat, skin them, and return the carcass to Sarah Woody for dissection at a later date.

![Figure 2. Map of Horicon Marsh that shows geographical collection sample points for Sarah Woody (Woody, 2022) and for muskrats caught in January 2023.](image)
The small and large intestines were retrieved from every muskrat. The intestines were individually placed in its own plastic zip lock bag, labeled, and placed in a -20°C freezer to be processed at a later time. When ready to be assayed, the intestines were set out in room temperature to thaw. A perimeter was set up to keep any spills contained. Within the perimeter worm were hooks, surgical scissors, pans, Nalgene wide mouth HDPE economy bottles, glass bowls, microscope slides, and plastic trays. In addition, sieves of different mesh count (Size 100, 40, 20), DI water, 80% and ethanol where also placed within arm’s reach. Once the intestines were thawed, the very first step was to untangle the intestines. All of the connective tissue keeping the intestines attached to one another was carefully cut to slowly release the intestines. Once untangled, the intestines were laid out in a straight line so that both ends of the intestines could be seen. The junction between the intestines was approximated and the intestines were split into small and large intestine. The intestines were once again laid down in a straight line and one end of the intestine was found. Scissors were carefully used to part the tissue like a book. It is imperative that one makes the incision by sliding the scissors through the intestines, and not by slicing through because the cutting motion performed with the scissors can damage any parasites within the intestinal walls. Once the intestines were fully open, a microscope slide was used to scrape the entirety of the intestines. Slight pressure was applied as the slide glided through the entire length of the intestines using the short side of the slide. Once the intestines were fully scraped, the microscope slide was washed by spraying it with DI water. This was performed over the plastic pan to allow the contents remaining on the slide to fall within the dish. The sediment recovered was washed
through two different sieve mesh counts (40 and 80 count). The sediment was washed three times, which allowed it to go from a murky brown color to a cloudy white color. All three washes were kept and inspected under a dissecting microscope for the presence of parasite. The remaining sediment was then poured into a 12-ounce milkshake glass, and decanted so any additional debris floated to the top and could be removed. Every cycle of decanting only takes three to five minutes and a minimum of one inch of water was replaced every time. Once the sediment slushy ran clear, the sediment was allowed to settle to the bottom of the milkshake glass before removing any surplus water. The intestines were subsequently placed in a plastic 32-ounce Nalgene bottle with DI water, shaken vigorously, and allowed to sit. The wash from the Nalgene bottle was also poured into a milkshake glass. The same decanting process was followed with the mixture retrieved from the Nalgene bottle. The intestines were removed and set aside so they could be observed under a scope at a later time. The contents of the milkshake glasses were slowly poured into glass bowls and the sediment was inspected under a dissecting microscope. A worm hook was used to fish out any and all parasites found within the mixture. The worms recovered were separated based on visual differences and placed into glass vials containing 80% ethanol along with a label that contained location information. The plastic tray was sprayed down with DI water and also examined through a dissecting microscope just in the event that any worms were stuck to the tray. The ethanol vials containing the worms were stored in a -20°C freezer until ready for molecular and morphological identification. From the 45 muskrats, a total of 4,704 intestinal parasites were recovered from the small and large intestines of the rodents.
Three trematodes, one nematode, and one cestode species made up the sum of the samples collected.

**Morphological Identification**

Two worms from every species except for the whipworm was fixed in 10 percent formaldehyde previous to being shipped to Southern Illinois University (SIU). The whipworms were mounted on slides with glycerin and taken along to the laboratory of Dr. Francisco Agústín Jimenéz Ruiz at Southern Illinois University at Carbondale for identification. Dr. Jimenéz has a standard operating procedure in place to stain flatworms, and that procedure was used to stain and mount the fixed trematodes and cestodes. Briefly, 70% ethanol with two to three drops of Carmine stain was prepared. Each worm species was dropped into the mixture and allowed to sit in the solution overnight. The next day the worm was washed in 70% ethanol, which helped in the release of excess stain and was only done for a maximum of one minute. A second wash was prepared with 70% ethanol with two to three drops of HCl to destain the specimen. When the specimen acquired a light pinkish color, it was removed from the wash. A third wash of 70% ethanol with two to three drops of NH$_4$OH was prepared and the specimens were allowed to sit in the solution for 15 minutes. Each specimen was then dehydrated. This was performed through a series of ethanol washes. Each worm was placed in 70% ethanol for 1 hour followed by a wash of 85% ethanol. The next two washes were both in 95% ethanol for 30 minutes. The very last wash was in 100% ethanol for one hour. The next step was to let the specimens sit in terpineol overnight, making sure that the specimen was fully submerged in the thick chemical. The next day, the worms were removed from
the terpineol and transferred to a small petri dish of xylenes. The last step was to mount the worms on an individual slide using Canada Balsam mixed with xylenes. Once mounted, the slides were set flat on a heating plate and allowed to dry. The mounted slides were observed under a microscope and with the use of the Yamaguti book series (1958), diagnostic keys were used to identify present morphological features in each worm.

**Molecular Assay**

We used standardized molecular assays to verify our morphological identifications. The workflow involves making a voucher for every specimen. One nematode, three trematodes, and one cestode were prepared for DNA extraction. It is important to note that only morphologically unimportant portions of each worm were harvested for DNA isolation. DNA was isolated from each worm piece using a commercial kit (DNeasy, Qiagen, Germantown, MD).

PCR was implemented to amplify target loci for the molecular identification of the five worm species. The amplicons were run on a gel before being sent out for sequencing to McLab laboratories. The concentrations of the gels were prepared at 1%. Twenty milliliters of 50X TAE solution were added to 980 Ultrapure water: this makes TAE buffer at 1X. To make the gel, 0.5 g of agarose was added to 50 ml of 1X TAE buffer. The mixture was microwaved for 30 to 60 seconds and the solution was poured into the gel tank. The wells were loaded with the amplicons and ran for 40 minutes at
120volts. The samples were then sent out for sequencing of amplicons (McLab, https://www.mclab.com/).

<table>
<thead>
<tr>
<th>Parasite species</th>
<th>Loci</th>
<th>Primers (Ribosomal)</th>
<th>Primers (Mitochondrial)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Quinqueserialis quinqueserialis</em></td>
<td>NDA1</td>
<td>N/A</td>
<td>NDA1: JB11, JB12</td>
</tr>
<tr>
<td><em>Plogorchis proximus</em></td>
<td>NDA1</td>
<td>N/A</td>
<td>NDA1: JB11, JB12</td>
</tr>
<tr>
<td><em>Echinostoma spp.</em></td>
<td>NDA1</td>
<td>N/A</td>
<td>NDA1: JB11, JB12</td>
</tr>
<tr>
<td><em>Cestode spp.</em></td>
<td>COI, 28S</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*Table 2.* This table presents the loci and the primers for both ribosomal and mitochondrial genes.

**Results**

Preliminary species identification presented in Table three are based on morphological identification.

<table>
<thead>
<tr>
<th>Location</th>
<th>Parasite1</th>
<th>Parasite2</th>
<th>Parasite3</th>
<th>Parasite4</th>
<th>Parasite5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teal</td>
<td>108</td>
<td>336</td>
<td>1</td>
<td>441</td>
<td>0</td>
</tr>
<tr>
<td>MPS</td>
<td>345</td>
<td>336</td>
<td>19</td>
<td>138</td>
<td>1</td>
</tr>
<tr>
<td>Radke</td>
<td>38</td>
<td>994</td>
<td>16</td>
<td>156</td>
<td>1</td>
</tr>
<tr>
<td>MPN</td>
<td>1077</td>
<td>530</td>
<td>7</td>
<td>159</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>1564</td>
<td>2196</td>
<td>43</td>
<td>894</td>
<td>3</td>
</tr>
</tbody>
</table>
### Table 3. Prevalence of five intestinal parasite species found within 45 muskrats in Horicon Marsh, Wisconsin.

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>28.9%</th>
<th>97.8%</th>
<th>33.3%</th>
<th>93.3%</th>
<th>6.7%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean intensity</td>
<td>120</td>
<td>49.9</td>
<td>2.86</td>
<td>20.7</td>
<td>1</td>
</tr>
</tbody>
</table>

Likewise, the prevalence of each species can be seen in table three. *Quinqueserialis quinqueserialis* had the highest prevalence at 97.8% and the *Cestode* spp. had the lowest at 6.7%. The highest mean intensity belonged to *Plagiorchis proximus* at 120 worms per infected individual, and the *Cestode* spp. was the lowest once again with a mean intensity of one per infected individual.

Morphological identification was conducted using the following parameters.

*Quinqueserialis quinqueserialis* is a flat translucent / white intestinal parasite that has characteristic wart-like papillae. Each species of the parasite can have different papillae arrangements which can aid in identification. *Q. quinqueserialis* has five rows of papillae with 16 to 18 papillae per row. The vitelline glands were anterior to the testis, and the cirrus sac was in the anterior portion of the body.
Figure 3. Image of the trematode *Quinqueserialis quinqueserialis* retrieved from the intestines of a muskrat from Horicon Marsh, Wisconsin.

*Plagiorchis Proximus* is a tear shaped trematode with oblique testis, meaning that they are diagonal to each other. This trematode has a muscular oral sucker and a ventral sucker that is positioned between the 1\textsuperscript{st} and 2\textsuperscript{nd} quarters of the body. The ovaries in this worm are positioned ventral to the ventral sucker. Lastly, the vitelline glands will be very prominent and are visible around the periphery of the worms as they are very voluminous.

![Image of Plagiorchis Proximus](image_url)

Figure 4. Image of the trematode *Plagiorchis proximus* retrieved from the intestines of a muskrat from Horicon Marsh, Wisconsin

The last trematode, *Echinostoma* spp., was not able to be identified morphologically but was narrowed down to two species of *Echinostoma* spp. The two species are

*Echinostoma trivolvis* and *Echinostoma revolutum*. Molecular identification would be needed in order to identify this trematode.
The nematode *Trichuris opaca* was identified based on spicule measurements for males and measurements between the distance of the vulva and esophageal – intestinal junction for females. The full length of the spicule was between 1.218mm and 1.398mm. The total length of the spicule sheath was between 0.241mm and 0.327mm in length. For female *Trichuris opaca* the distance between the vulva and the esophageal – intestinal junction ranged from 0.91mm and 0.103mm. Eggs were present in one of the female *Trichuris* worms and measurements were taken of the eggs. The mean measurement of a *Trichuris* egg was 0.030mm x 0.015mm.
An unidentified *Cestode* spp. was recovered from the intestinal tracts of the muskrat. The tapeworms were recovered in small segments with no trace of the scolex. Morphological identification was not possible because of the lack of scolex. Although the species of the cestode was not identified, I concluded that this was a non-taeniid cestode because of the lack of post ovarian vitelline glands. The gravid segments of this cestode spp. were short and very wide. Some of the segments had visible ovaries lateral to the midline of the worm body.

**Molecular Analysis of Parasite DNA**

The genetic barcoding was received for four out of the five worms. The sequences were run through the NIH BLASTN software nucleotide database. This was done by performing a nucleotide sequence query to search for worm genetic matches. The sequences for 18s, CYTB, ITS, and COX1 of the *Trichuris* spp. were ran through the program and identified the nematode as *Trichuris arvicolae*. The previously unknown *Echinostoma* spp. had the NAD1 mitochondrial gene amplified. When the sequence for
the gene was blasted, the trematode was identified as *Echinostoma trivolvis*. The sequence for the *Quinqueserialis quinqueserialis* NAD1 sequencing data identified the flatworm as *Notocylus intestinalis*. Lastly, the sequencing data for the trematode *Plagorchis proximus*, also via the NAD1 gene, identified the worm as *Metorchis bilis*. No genetic barcode data were provided for the unidentified cestode as of the writing of this thesis.

*Quinqueserialis quinqueserialis*

Eighty Eight percent shared identity with the closest match, *Notocotylus intestinalis*. The identification was performed using the NADH dehydrogenase subunit 1. The data are not shown in this section, this due my data being the first to be submitted for the parasite. The identity of the parasite has been confirmed via morphology, and the sample will be deposited in the Manter Parasite Museum.

*Plagiorchis proximus*

The NADH dehydrogenase subunit 1 revealed a 77% shared identity. The closest related match is *Alloglossidium* spp. The data are not shown in this section for this parasite. The data in this study will be the first to be submitted for the parasite *Plagiorchis proximus*. The identity of the parasite has been confirmed through morphology and the specimen will also be deposited in the Manter Parasite Museum.

*Echinostoma revolutum*
The NADH dehydrogenase subunit 1 amplicon received from MacLab laboratories was compared to the Nebraska Detweiller reference from the NIH database. There was a 100% identity match to *Echinostoma trivolvis*. However, morphologically the parasite has been identified as *E. revolutum*. This parasite will be deposited in the Manter Parasite Museum.

**Figure 8.** NADH dehydrogenase subunit 1

*Trichuris opcaca*

*Trichuris opaca* had identity matches to *Trichuris* spp. The closest relative was *Trichuris arvicolae* but no definitive matches were recovered. The mitochondrial cytochrome oxidase 1 gene was amplified along with the 18s rRNA and ITS1 rRNA genes.
Figure 9 Panel A. Internal transcribed region 1 of rDNA
Figure 9 Panel B. 18s RNA
Discussion

In this study, every single muskrat was infected with at least one species of parasite. The lowest prevalence came from the *Cestode* spp. and the highest from the *Quinqueserialis quinqueserialis* trematode. There was some discrepancy in the results between morphology and molecular identification. When sequences for *Plagorchis proximus*, *Quinqueserialis quinqueserialis*, and *Trichiurus opaca* were run through the NIH BLAST database, the sequences did not produce a 100% similarity match. For *Trichiurus opaca*, multiple gene sequences were BLASTED which indicated the organism belonged to a *Trichuris* spp. (18s, ITS, CO1, CYTB, 28s). For all trematode species the NAD1 gene was amplified initially but this region alone was not enough to identity each individual worm to the species level. More loci would need to be amplified in order to narrow down
the species of each trematode. This could be a great opportunity as the absence of an exact match to these amplified regions could signify this exact genetic information has not yet been made available in the database. This makes for an opportunity to contribute unique genetic information of muskrat intestinal helminths.

Overall, this project could help not only the marsh and its fauna but also the public and trappers that are there from year to year. The *Echinostoma trivolvis* identified is infective to humans and it does cause some disruption in the intestinal wellbeing of humans. The parasite travels to the intestines of the individual and latches on to the mucosa of the intestines causing catarrhal inflammation (*Echinostomiasis*, 20119). It is also necessary to investigate further what species of cestode was found in the intestines of the muskrat in the off chance that the cestode too is infective to humans. As for the Trichuris, whether it be *Trichuris opaca* or *Trichuris arvicolae*, preventing infection will be rather difficult. The eggs of the whipworm are passed on through feces, thankfully these worms only infect rodents and not humans (Deter et al., 2009; Ganoe et al., 2020). If the health of the muskrat population at any point is in jeopardy, perhaps the removal of snails, much like how it was done for the prevention of schistosomiasis can be performed (Moloo, 2020). The trematodes in this study have snails as primary and secondary hosts. It would be up to the marsh to assess whether the intervention was beneficial and worthwhile.
Chapter IV

Conclusion

Some of the muskrats were more heavily parasitized that others which brings up the question of why. Flowing bodies of water, lotic waterways, have high species richness and most of it can be seen downstream. Many of the parasites in lotic systems require the help of the water currents to reach their desired hosts. The movement of the water in these lotic systems allows for higher species richness and diversity (Paterson et al., 2018). The marsh can be considered a lentic water system. The water stays in place in the ponds with little movement since there isn’t much outflow. Muskrats can continuously expel eggs or proglottids into the water via its feces resulting in continuous infection. If lentic water systems are opposite to lotic, in the sense of parasite diversity and richness this could explain the limited diversity in marsh muskrats.

Overall, the goal of this project was to provide insight on the intestinal parasites of the muskrat. Through my research I was able to establish the presence of five parasites in total. Three trematodes identified morphologically as Plagiorchis Proximus, Quinqueserialis quinqueserialis, and Echinostoma spp. The most prevalent parasite was Q. quinqueserialis at 97.8%, followed by Echinostoma spp. (93.3%), Trichuris opaca (33.3%), Plagiorchis proximus (28.9%), and Cestode spp. (6.7%). The mean intensity was as follows from highest mean intensity to lowest; Plagiorchis Proximus (120), Q. quinqueserialis (49.9), Echinostoma spp. (20.7), Trichuris opaca (2.86), Cestode spp. (1). Quinqueserialis quinqueserialis was present in all but one muskrat. They plagued the large intestines and at times it was hard to understand how that many flat worms could be
present in such tight a space. *Echinostoma* spp. were present in all but three of the muskrats. While this trematode was not present in the same intensity as *Q. quinqueserialis*, its size made up for quantity. One individual was the exception as it was infected with 315 *Echinostoma* spp. The intestinal tract of this muskrat was quite literally bursting with coiled worms, as they were tightly packed in the small intestine. Very few washes had to be performed in order to tell the degree to which this muskrat had been parasite by this worm. *Plagiorchis proximus* was present in fewer individuals, but when it was present many individuals were recovered. This drove up the mean intensity even though the prevalence was low. Two muskrats had over 400 trematodes present. Although small, the overwhelming numbers made up for the lack of mass. The remaining two parasites, *Trichurus opaca* and the *Cestode* spp. were found in much smaller number. The cestodes were recovered from the full length of the small and large intestines, however, they were recovered in pieces. The male *Trichurus* spp., on the other hand, was quite striking as it had its reproductive organs on full display.

Moving forward, further research should be conducted to cover other aspects of parasitism. In this study I was unable to get fecal samples and blood samples from the muskrats obtained. Observing fecal samples for the presence of eggs and blood smears for the presence of blood flukes could reveal more about the parasites that inhabit the muskrat. The collection of ectoparasites like mites or ticks would provide information of bacterial and viral infections. A full understanding of all parasitism affecting the muskrat could aid in further understanding why the decline in muskrat population is occurring.
This in conjunction with efforts from conservation and environmental biologists could prevent the local and global decline of the aquatic rodent.
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Tularemia --- United States, 1990–2000. (2002). CDC. Retrieved 8/17/23 from [https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5109a1.htm#fig1](https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5109a1.htm#fig1)


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ADDENDUM: Parasites reported from the common muskrat

<table>
<thead>
<tr>
<th>Class</th>
<th>Species</th>
<th>Location</th>
<th>Mode of Infection</th>
</tr>
</thead>
</table>
| Protozoa    | *Giardia* spp          | • Microvillus of small intestine  
                  | 1. Duodenum  
                  | 2. Jejunum  
                  | • Cysts in Feces  
                  | • Trophozoites in Intestinal smear | Direct infection from cysts in water  
                  |                                                                       | • Temperature important in viability of cysts |
| Protozoa    | *Cryptosporidium* spp  | • Microvillus of Intestines  
                  | 1. Ileum  
                  | 2. Colon  
                  | • Oocyst in feces Intestinal Smear | Direct infection from cysts in water  
                  |                                                                       | • Temperature important for viability of cysts |
| Trematode   | *Alaria mustelae*      | • Cysts can be found throughout the body (in tissue).  
                  |                                                                       | 1. Eggs are released from the definitive hosts and miracidia hatch from the eggs.  
                  |                                                                       | 2. The miracidia infect snails. The cercaria are then released from the snails and infect frogs and tadpoles.  
                  |                                                                       | 3. The muskrat will then eat the host and get infected to become the metaparatenic host |
| Trematode   | *Allossogonoporus marginalis* | • Small intestine | Life cycle is not known, but the muskrat is thought to be the definitive host |
| Trematode   | *Catatropis fimbriata*  | • Found in the duodenum | This parasite is an avian parasite (muskrat is possibly an accidental host) – The muskrat counterpart is Notocotylus sp.  
                  |                                                                       | • Based on other species, snails are intermediate hosts |
| Trematode   | *Echinostomum armigerum*| • Elliptical flattened body can be found in the duodenum | 1. First intermediate host is a snail  
                  |                                                                       | 2. Second intermediate host is potentially fish |
| Trematode   | *Echinostomum callawayensis* | • Can be found in the duodenum | 1. First intermediate hosts snails  
                  |                                                                       | 2. Secondary hosts have not been identified  
<pre><code>              |                                                                       | • The muskrat is thought to be the definitive host |
</code></pre>
<p>| Trematode   | <em>Echinostomum coalitum</em> | • Can be found in the duodenum | As per other species of echinostoma, the muskrat would most likely be infected via the ingestion of freshwater snails |
| Trematode   | <em>Echinoparyphium contiguum</em> | • Can be found in the duodenum | When considering other species, the muskrat will likely get the infection from freshwater pulmonate snails (i.e. <em>Stagnicola elodes</em>, <em>Radix balthica</em>, <em>Myxas glutinosa</em>) |
| Trematode   | <em>Echinostoma recolutum</em> | • Small intestines | 1. First intermediate hosts are planorbid snails (Lymnaea elodes) |</p>
<table>
<thead>
<tr>
<th>Trematode</th>
<th>Echinostoma trivolvis</th>
<th>• Small intestine 1. Adults live in the small intestine 2. Metacercaria excyst in duodenum</th>
<th>1. First intermediate host are pulmonate snails 2. Infection after eating secondary host, a snail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trematode</td>
<td>Echinostoma revolutum</td>
<td>• Small intestine</td>
<td>1. Infection happens after eating gastropods • Fingernail clams • Snails</td>
</tr>
<tr>
<td>Trematode</td>
<td>Echinococchus schwartzi</td>
<td>• Small intestine</td>
<td>1. Snails are the first intermediate host 2. The second intermediate hosts are fish</td>
</tr>
<tr>
<td>Trematode</td>
<td>Echinoparyphium recurvatum</td>
<td>• Small Intestines</td>
<td>1. Infection happens after eating infected snails</td>
</tr>
<tr>
<td>Trematode</td>
<td>Fibricola crater -Previously Hemistomum cratera</td>
<td>• Can be found in the duodenum and Caecum</td>
<td>1. First intermediate host are pulmonate snails 2. Secondary hosts are not known, but thought to be tadpoles • Muskrats are one of many definitive hosts</td>
</tr>
<tr>
<td>Trematode</td>
<td>Notocotylus filamentus</td>
<td>• Large Intestine 1. Caecum</td>
<td>Life cycle unknown • Other species have a snail as intermediate host</td>
</tr>
<tr>
<td>Trematode</td>
<td>Notocotylus urbanensis</td>
<td>• Small intestine</td>
<td>1. First intermediate host are pulmonate snails 2. Second intermediate hosts are also pulmonate snails</td>
</tr>
<tr>
<td>Trematode</td>
<td>Nudacotyle Novicia</td>
<td>• Large Intestine</td>
<td>1. Cercariae encyst in vegetation and infection occurs when eaten</td>
</tr>
<tr>
<td>Trematode</td>
<td>Opisthorchis tonkae</td>
<td></td>
<td>1. First intermediate host are snail 2. Secondary host is the sand shiner(fish)</td>
</tr>
<tr>
<td>Trematode</td>
<td>Plagiorchis noblei</td>
<td>• Intestine</td>
<td>1. First intermediate host are pulmonate snails 2. Secondary host are dipteran larvae • Mammals are usually not part of the cycle they are accidental hosts</td>
</tr>
<tr>
<td>Trematode</td>
<td>Plagiorchis Proximus</td>
<td>• Small Intestine 1. Duodenum</td>
<td>1. First intermediate host are snails 2. Infection occurs from eating infected chironomids • Dragonfly • Mosquito larvae • Mayfly naiads</td>
</tr>
<tr>
<td>Trematode</td>
<td>Phagicola lageniformis</td>
<td>• Intestines</td>
<td>1. Commonly infection found in fish</td>
</tr>
<tr>
<td></td>
<td>Name change &gt;possibly Ascocostyle agrense</td>
<td>Large Intestine 1. Caecum</td>
<td>Life cycle unknown 1. Other species form cercariae in vegetation</td>
</tr>
<tr>
<td>----------------</td>
<td>------------------------------------------</td>
<td>----------------------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Trematode</td>
<td>Pseudodiscus zibethicus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trematode</td>
<td>Ptyalincola ondatrae</td>
<td>Mouth and Salivary glands</td>
<td>1. First intermediate host is unknown 2. Secondary host are unionid mussels</td>
</tr>
<tr>
<td>Trematode</td>
<td>Quinqueserialis quinqueserialis -Also referred to as (Notocotyle quinquiseriale)</td>
<td>Large Intestine 1. Caecum</td>
<td>1. First intermediate host is the snail (Gyraulus parvus) 2. No second intermediate host – the cercariae leave the first intermediate host and encyst in aquatic vegetation which the muskrat will eat One of the most commonly found within Muskrats</td>
</tr>
<tr>
<td>Trematode</td>
<td>wardius zibethicus</td>
<td>Colon and Caecum</td>
<td>1. First intermediate host is the fresh water snail (Helisoma antrosum) contain the rediae. 2. The metacercariae encyst in aquatic vegetation</td>
</tr>
<tr>
<td>Trematode</td>
<td>Schostosomatium douthittii</td>
<td>Intestinal tract</td>
<td>1. Miracidia acquired in the water.</td>
</tr>
<tr>
<td>Trematode</td>
<td>Ascaris spp</td>
<td>Intestinal tract</td>
<td>Direct life cycle 1. Ingested directly from the environment when the eggs are released via the feces</td>
</tr>
<tr>
<td>Nematode</td>
<td>Capillaria hepatica</td>
<td>Cysts found in the liver</td>
<td>1. Cyst can only hatch when they are exposed to air (oxygen). Eggs released via the feces Oxygen releases the nematode and are ingested by the muskrat</td>
</tr>
<tr>
<td>Nematode</td>
<td>Capillaria michiganesis</td>
<td>Intestinal tract</td>
<td>1. Eggs are passed in the feces and hatch in moist environments. 2. Muskrats ingest the nematode This species is muskrat specific</td>
</tr>
<tr>
<td>Nematode</td>
<td>Capillaria ransomia</td>
<td>Cyst can be found in the liver Adult can be found in the duodenum</td>
<td>Live cycle Unknown</td>
</tr>
<tr>
<td>Nematode</td>
<td>Litomosoides carinii</td>
<td>Intestine</td>
<td>1. adult muskrat will often feed on the cotton rat Filarial parasite of the cotton rat</td>
</tr>
<tr>
<td>Nematode</td>
<td>Longistriata dalrymplei</td>
<td>Intestine</td>
<td>1. Muskrat will eat a rat and become infected Parasite of Rattus sp.</td>
</tr>
<tr>
<td>Nematode</td>
<td>Rictularia ondatrae</td>
<td>Intestine</td>
<td>1. adult muskrat will often feed on the cotton rat</td>
</tr>
<tr>
<td>Class</td>
<td>Species</td>
<td>Location</td>
<td>Mode of Infection</td>
</tr>
<tr>
<td>-------------</td>
<td>--------------------------------</td>
<td>----------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Nematode    | *Strongyloides ratti*          | Small Intestine, Eggs in the feces | 1. Eggs hatch in soil when the environment is moist or when in water  
2. Infected by ingestion or through penetration of the parasite via the skin |
| Nematode    | *Trichostrongylus fiberius*    | • Can be found in the duodenum and caecum | Life cycle is not known, but other species of this species have a direct life cycle |
| Cestode     | *Anomotaenia telescopia*      | • Small intestine  
1. Duodenum | The life cycle for this parasite is unknown and the infection of muskrats might be accidental. In other species the first intermediate host are insects and the definitive hosts are birds |
| Cestode     | *Hymenolepis evagina*         | • Small Intestine  
1. Duodenum | 2. Intermediate host are the fresh water ostrocod (*Cyclocypris laevis*) |
| Cestode     | *Hymenolepis taeniaeformis*   | • Eggs in feces | 2. Infection through ingestion of embryonated  
1. Eggs released in the feces of infected animals |
| Cestode     | *Taenia taeniaeformis*        | • Cysts are found in the liver | 1. The muskrat is the intermediate host  
2. Felines are the definitive hosts  
• Muskrats can also harbor adult cestodes but they become dead end hosts |
| Palaeacanthocephela | *Corynosoma spp* | • Eggs in feces  
• Adults in small intestine | 1. Intermediate hosts are insects.  
2. Eggs are shed in the feces of the infected host.  
3. The definitive host will become infected when it ingests the infected insect  
• Common species infecting aquatic mammals include:  
  1. *C. australe*  
  2. *C. cetaceum* |
| Acanthocephela | *Polymorphus spp.* | • Intestinal tract | 1. Infected from intermediate host which is an amphipod |

Table 1. This table gives names of common intestinal parasite of the muskrat, their location, and mode of infection (Barker, 1914; Bitto & Aldras, 2009; Ganoe et al., 2020; Lapp, 2013; Meyer & Reilly, 1950; Rice & Heck, 1975; Zabiega, 1996)