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Assessing Gastropods as Parasite Vectors on Small Ruminant Farms

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ASSESSING GASTROPOD PARASITE VECTORS ON SMALL RUMINANT FARMS

By

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By Rachel E. White

Dissertation Advisor: Dr. Anne Lichtenwalner

An Abstract of the Dissertation Presented in
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An animal health challenge that many small ruminant producers face is the impact of parasites on production, such as weight loss, reproductive problems, and product yield. The nematode, *Parelaphostrongylus tenuis* (meningeal worm) can cause severe neurologic symptoms and death in small ruminants. This "phantom parasite" does not have a premortem diagnostic test to detect it, thus the level of incidence in livestock is unknown. Because of this, prevention is the best option to limit incidence, though no research on *P. tenuis* risk factors to livestock naturally on grazing pastures has been explored. This work aimed to investigate the transmission risk factors of *P. tenuis* through the intermediate hosts, terrestrial gastropods (snails and slugs) and to explore methods of mitigating risk on a pasture-wide scale.

Over two years, collections and surveys of gastropods were held on six small ruminant farms. This study found that 1% of gastropods living on pastures carry *P. tenuis*. An in-depth analysis of gastropod intermediate hosts showed a variety of helminths in addition to *P. tenuis*, including *Muellerius capillaris* or sheep/ goat lungworm. Snail characteristics, such as shell size, and seasonality on the pasture were correlated with *P. tenuis* larvae in infected snails.

To investigate methods of controlling high abundances of gastropods on pasture, an integrated pest management approach was explored through an on-farm case study. Pastured

poultry, using chickens, and mowing were both found to be successful practices for mitigating snail populations on pastures. However, in plots intentionally allowed to regrow, snail populations quickly rebounded, suggesting that mowing is a practice that may need to be maintained to be effective.

The knowledge, attitudes, and management practices were examined by stakeholders to determine risk perceptions of *P. tenuis* and other parasites. The farmers involved with this study and veterinarians reported changes in knowledge after learning about study results, and voiced challenges and needs based around animal health education and information gathering.

These data provide insight into the factors of risk and risk-reduction of *P. tenuis* to small ruminants on grazing space. Farmers and managers may consider these practical methods for animal health improvement and prevention.

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CHAPTER 1. INTRODUCTION

1.1 One Health paradigm

This research investigates animal, environmental, and anthropogenic conditions that affect the unique pathogen-host transmission involving gastropods. The interrelatedness of these groups forms the conceptual framework of One Health (Figure 1). The Centers for Disease Control and Prevention defines One Health as "a collaborative, multisectoral, and transdisciplinary approach working at the local, regional, national, and global levels with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment" (CDC, 2018). Several important health topics formed the genesis of the One Health concept, including vector-borne diseases and parasite infections. Worldwide, zoonotic diseases account for 75% of emerging infectious disease and an estimated 17% are thought to be vector-borne (Faburay, 2015). A One Health transdisciplinary approach to vector-borne and zoonotic diseases is important to the understanding and management of these "wicked" problems.

Many factors motivating interactions between people, animals, and the environment have led to the spread of zoonotic diseases. The emergence and reemergence of parasitic diseases is partly attributed to climate related factors and events (Short et al., 2017). Warming climate has changed pathogen transmission dynamics by altering parasite life stages, phenology, movement, and behavior (Altizer, et al., 2013). Climate change, coupled with changes in land use, has altered species distributions across the landscape including vector species (Pecl et al., 2017). Human population growth has increased development and deforestation, reducing natural habitat and biodiversity while encroaching closer to wildlife. This positions people to experience

increased risk of spillover events from wild animals (Thompson, 2013). Movement of people, animals, and animal products have also facilitated the spread of disease (Gianelli et al. 2016).

Agriculture is a unique area within the scope of One Health where farmers, domestic and wild animals, and the surrounding environment are interconnected. The close proximity to one another can result in unintentional disease spillover. Additionally, the farm animal industry largely contributes to antibiotic and multi-drug resistant microbes, a major One Health concern (One Health Initiative, 2021; Muloi, 2019; CDC, 2018). An interdisciplinary approach has led to surveillance and management efforts for controlling zoonotic diseases such as brucellosis, bovine tuberculosis, *Escherichia coli* and other food-borne bacteria (Dadar et al, 2021; Mohamad, 2020; Ludden et al, 2019; Franco et al, 2014). As human populations grow, the need for sustainable solutions for wicked problems are important for the health of people, animals, and the environment.

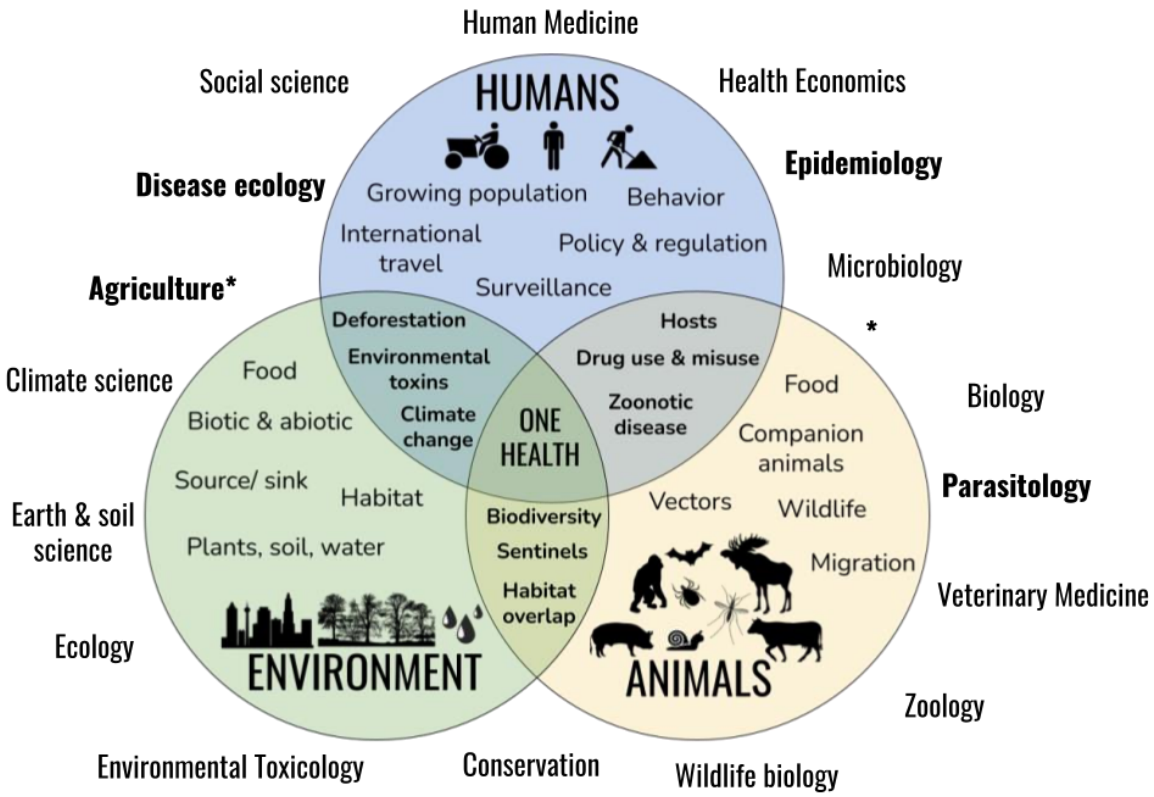

Figure 1. One Health framework of interconnected fields, actions, and stressors. Adapted from the 'One Health Umbrella' developed by One Health Sweden and the One Health Initiative (One Health Initiative, 2021).

1.2 Gastropods as vectors

Invertebrate vectors for pathogens and parasites garner much research attention for their public and veterinary health implications, with mosquitoes and ticks being popular study subjects. Gastropods, or snails and slugs, are not to be overlooked as vectors for parasites causing important diseases. Gastropod-borne helminth diseases are of growing concern, affecting more than 300 million people globally (WHO, 2022). Gastropods can act as intermediate (secondary host for immature parasite stages) or definitive (primary host of parasite reaching

sexual maturity and reproduction) hosts for pathogens which can cause significant disease in humans and animals, including schistosomiasis, angiostrongyliasis, and fascioliasis.

Schistosomiasis, also known as bilharziasis, is a neglected tropical disease affecting predominantly low-income countries, and is prevalent in areas of Asia, Africa, and South America. In 2019, at least 236 million people required treatment worldwide (WHO, 2022). Areas of low sanitation and poor infrastructure, including clean water availability and medical resources, coupled with people who utilize natural water sources for agriculture, fishing, bathing, or swimming, face heightened risk of infection due to the life history of the parasite. Schistosomiasis, caused by flat worm trematodes (also identified as a blood fluke) in the genus *Schistosoma*, has a complex life cycle within aquatic environments. Definitive hosts can vary depending on species of parasite. For example, *Schistosoma japonicum*, can infect 46 different species of animals including horses, pigs, goats, cattle, dogs, and cats (He et al, 2001). The species *S. mekongi* and *S. mansoni* frequently infects dogs and primates. The parasite incorporates an asexual phase within snail intermediate hoses and a sexual phase in humans or other vertebrates (Xiao-Ting et al, 2018). Infection can cause severe disease in humans, with children being the most vulnerable. Different symptoms are observed depending on the different species throughout the known global regions with this disease. Prevention and control strategies have been used globally, including various biological and chemical methods. Sokolow et al (2016) examined large-scale control efforts of schistosomiasis in 83 countries over the past century. Their assessment of the "best" control strategies indicated that traditional snail mitigation methods of molluscicides and biological control, notably using non-native competitor snails, worked better at reducing disease than test-and-treat campaigns.

Rat lungworms, *Angiostrongylus cantonensis* and *A. costaricensis*, are of rising veterinary and public health concern as they can cause severe gastrointestinal or central nervous system disease in humans and animals, called angiostrongyliasis. In a 2008 report, *Angiostrongylus* impacted over 3,000 people from Southeast Asia, Australia, the Pacific Islands, and the Caribbean (Wang et al, 2008). Rat lungworm has recently been documented in the southern United States and Hawaii and where it has caused severe human illnesses (Kim et al, 2002; Stockdale-Walden et al, 2015; Flerlage et al, 2017). As with other gastropod-born helminths, the life cycle of *Angiostrongylus* is complex. Both worm species reproduce within rats and stage-one larvae are expelled through feces. Larvae are up taken into gastropod intermediate hosts through ingestion or direct penetration of tissue or through the slime layer. Over 160 gastropod species, including *Achatina spp., Biomphlalaria spp., Bulinus spp., Lymnaea spp., Pomaecea spp*, can become infected and develop stage-one larvae (CDC, 2019). Six gastropod species have been identified in the United States as intermediate hosts for *A. cantonensis* (Valente et al, 2020). The worm molts for two stages and develops into infective, third-stage larvae. The worm fulfills its lifecycle when a rat ingests an infected gastropod. Rats, other animals, or humans may ingest paratenic hosts that may have acquired the worm by eating a gastropod (Giannelli et al, 2015). These hosts (e.g., shrimp, crabs, toads and free-living flatworms) serve as transport vessels for the worm but do not facilitate growth of immature stage larvae. Dead end hosts include humans, and domestic and wild animals. Horses, dogs, swine, and cattle have been successfully infected under natural or experimental conditions (Costa et al 2000; Jindrak and Alicata, 1970; Jindrak and Alicata, 1968). *A. cantonensis* affects the digestive tract while *A. costaricensis* affects the central nervous system and brain. Angiostrongyliasis presents various symptoms such as vomiting, fatigue, headache, and neurologic disruption, and chronic

conditions such as eosinophilic meningitis, meningoencephalitis, and ocular angiostrongyliasis may occur (Xiao-Ting et al, 2018). Though these species are tropical, concern that climate change will fuel the distribution of this zoonotic parasite in the U.S. is growing (Stockdale-Walden et al, 2015; Flerlage et al, 2017).

Fascioliasis is caused by the liver fluke (trematode) *Fasciola hepatica*, also known as the common liver fluke and sheep liver fluke, and *Fasciola gigantica*. These parasites are found in wild and domestic ruminants, such as sheep and cattle. They are found world-wide in over 70 countries (CDCb, 2018). The life cycle of *F. hepatica* and *F. gigantica* is similar to those of other aquatic snail-borne helminths. Infected hosts, primarily ruminants which defecate in or near freshwater sources, introducing eggs, which then hatch into miracidia. Miracidia search for a suitable snail intermediate host where it then transforms into sporocysts, then rediae, and finally cercariae. The cercariae leaves the snail tissue to encyst on aquatic plant matter. When metacercariae on plant matter is ingested by a vertebrate host, the parasite travels out of the intestinal wall. They then migrate through the liver to the biliary ducts where they reproduce (CDCb, 2018). Gastropod hosts include snails of the genus *Lymnaea* (Nyirenda et al, 2019). In sheep and cattle, *Fasciola spp*. can cause stunted growth, reduced production, mortality, and poor scores in liver evaluation during meat inspection (Nyirenda et al, 2019). In animals and humans, damage to liver tissue and the bile duct are observed. Farmers can treat infected animals with anthelmintics, but they do not work as a preventative. Diagnosis via fecal examination under microscope might be confirmed if *Fasciola* eggs are seen.

Management strategies for gastropod intermediate hosts is an under-researched area in the human and animal health fields. Gastropod-borne diseases that affect humans and farmed animals are on the rise, likely due to climate change, the increased global movement of goods,

and invasive species spread (including invasive snails). Better understanding of the pathogenesis of gastropod-borne parasites within intermediate hosts is needed to inform managers and public health officials of methods to reduce the risk of these pathogens.

1.3 *P. tenuis* and host relationships

Parelaphostrongylus tenuis, also known as meningeal worm, is a debilitating parasite which causes neurological damage in wild and domestic animal aberrant hosts, such as moose (*Alces alces*) and elk (*Cervus canadensis*), as well as sheep (*Ovis aries*), goats (*Capra hircus*), camelids (*Lama glama* and *Lama pacos*), horses (*Equus caballus*) and cattle (*Bos taurus*) (Lankester, 2010; McIntosh et al, 2007; Pybus et al, 1996; Gutherey et al, 1979; Ismail et al, 2011; Mittelman et al, 2017; Duncan et al, 1998). While it does not often cause symptoms in its definitive host, the white-tailed deer (*Odocoileus virginianus*; WTD), it can be fatal to other mammals. Deer abundance, coupled with high prevalence of *P. tenuis*-infected gastropods, can cause spillover into aberrant hosts where ranges overlap. Agricultural systems in the Northeast often have observed overlap of snails and slugs, WTD, and farmed animals, which may increase risk of *P. tenuis* to livestock. When these aberrant hosts consume molluscs infected with meningeal worms, the likelihood of recovery without treatment is low and may lead to high rates of morbidity and mortality, causing farmers to alter management strategies.

Gastropod species serving as intermediate hosts for meningeal worm have been well documented. In 1968, Anderson and Lankester published a foundational study confirming several species of gastropods as intermediate hosts for *P.tenuis* (Lankester & Anderson, 1968). Almost 10,000 gastropods were examined in search of the parasite with about 4% being infected. In Maine, Gleich et al. (1977) examined forest dwelling gastropods in central Maine. He found

that 4%-19% of snails and slugs carried a range of nematodes, but *P. tenuis* was only found in *Pallifera* sp. slugs.

The lifecycle of the *P. tenuis* consists of an egg-laying adult within a WTD's venous sinuses and subdural space of the cranium (Anderson, 1963). Stage one larvae (L1) migrate through the bloodstream and reside in capillaries of the lungs. The larvae then enter bronchioles and move up the respiratory tract until they reach the pharynx. The larvae are coughed up and swallowed, then move into the digestive tract. L1 are then expelled within the mucus layer around WTD's feces and may be ingested by a gastropod (the intermediate host) wherein they develop into second- and third- stage (infective; L3) larvae. From the gastrointestinal tract, the larvae move into the spinal nerves and spinal cord, then migrate to the space surrounding the deer's brain where they grow to maturity. For the creation of new L1s, both sex adults must be present and in approximately 40 days, mature worms produce eggs and L1 are passed through the feces (Anderson, 2000). Adult worms can live and reproduce for up to six years within the deer host (Duffy, et al., 2004).

The distribution of meningeal worm is widespread throughout the United States wherever WTD are present. In Minnesota, Slomke et al. (1995) found adult *P. tenuis* present in 82% of sampled deer (311 individuals) of different age and sex classes. In Maine, Behrend and Witter's (1968) found an average of 84% *P. tenuis* prevalence in WTD. If an aberrant host ingests an infected gastropod, the life cycle of meningeal worm cannot be completed. The migration of the L3 into the central nervous system causes severe impairment of motor and neurological function. The symptoms of infection include ataxia, stiffness, circling, blindness, head-tilt, and loss of fear of humans (Anderson, 2000). These dead end hosts for the worm do not shed eggs, thus traditional diagnostics for parasites using fecal egg analysis isn't viable.

Diagnosis of *P. tenuis* is currently not easy or cost-efficient. Infection may be confirmed at necropsy via histologic evaluation of the entire spinal cord and brain of the animal, but even careful searching may fail to show signs of the parasite (Anderson and Prestwood, 1981). Genetic testing has been studied since the 1990s more recent breakthroughs in diagnostic approaches in the late 2010s. Attempts at making a serodiagnostic assay from antibodies for *P. tenuis* started in 1992 with Dew, et al., followed by Duffy et al., 1993; Neumann et al., 1994; and then Bienek et al., 1998. These early tests were not definitive in their diagnosis, as the testing often identified other genuses such as *Dictyocaulus* which created false positives (Neumann et al., 1994; Bienek et al., 1998). In 1999, Ogunremi et al. found the first unique *P. tenuis* larval antigen and created an enzyme-linked immunosorbent assay (ELISA). This method was recreated and altered to test within white-tailed deer, moose, and elk (Ogunremi, et al., 1999; Ogunremi et al., 2002a; Ogunremi, et al. 2002b). Soon after, complementary DNA libraries became available for adults (Duffy et al., 2002) and larvae (Duffy et al., 2006). In 2008, Ogunremi et al., created a complementary DNA expression library to an ELISA by reverse mRNA transcription of P. tenuis adults. More recently, development of a serological assay for *P. tenuis* in moose shows promise for the advancement of veterinary diagnostics for the worm (Richards et al., 2023).

No known meningeal worm cases in humans have ever been recorded, though similarities with the rat lungworm (*A. cantonensis*), which causes severe meningitis and shares a strikingly similar lifecycle to *P. tenuis*, may raise considerations on if humans can contract *P. tenuis*. Rat lungworm has been documented throughout the southern United States and Hawaii where it has caused human illnesses (Kim et al, 2002; Stockdale-Walden et al, 2015; Flerlage et al, 2017). In horses, a monogastric species, both strongyloid nematodes (*A. cantonensis* and *P. tenuis*) have

been documented cohabitating the brain or spinal cord region (Costa et al, 2000; Tanabe et al, 2010). Safe food handling by washing fruits and vegetables will help prevent accidental consumption of gastropod-borne parasites.

1.4 Small ruminant parasite management

Internal parasites of livestock are a widespread problem that restricts production of animal products and effects the economy. An assessment of the economic burden of parasitic helminth infections to the ruminant livestock industry in Europe estimated that the annual cost, including deaths and treatment financial estimates, was over two billion U.S dollars (Vercruysse et al, 2018). The development of helminth disease control strategies is an ongoing effort worldwide as new pathogens emerge, and traditional parasites evolve. The frequent use of anthelmintics in livestock industries has caused the emergence of multiple drug-resistant parasites. For many farmers, increasing production (e.g., milk output, weight gain, and wool growth) is a primary objective to enhance profits. The presence of parasites within livestock can reduce food intake and metabolic processes (e.g., protein absorption) which affects production (Charlier et al, 2018). Parasites can cause direct tissue damage (e.g., liver cysts/ lesions) which limits marketability, thus decreasing profit. Additionally, the cost of sick animals due to parasites (e.g., treatment costs, reduced reproductive output) may disrupt producer bottom line, especially for farmers with small profit margins. Financial losses due to mortality, particularly in immunocompromised animals, can have significant impact on overall herd or flock profitability. Implementing methods such as pasture management, rotational grazing, strategic deworming, and genetic selection can help mitigate the impact of parasites (Grenfell, 1988; Vercruysse et al., 2020; McManus et al., 2014).

Historically, fecal egg counts (FEC) have been the dominant method and a proxy for parasite burden and recounts can help determine chemical resistance in gastrointestinal worms (Morris et al, 1997). This can be performed by using Modified McMaster, Triple Chamber McMaster, Mini-FLOTEC, which all have varying sample weights, flotation solution, centrifugation, chambers, and precision (Boareki et al, 2021). Packed cell volume count (PCV) is a method to detect percent of red blood cells in blood; a PCV of below 20 is typically a symptom of bloodsucking parasites (i.e., *Haemonchus contortus)*. A popular method farmers often use in tandem to FEC, is the FAMACHA method. FAMACHA scores allow farmers to estimate the level of anemia caused *H. contortus* in small ruminants by matching the color of the eye mucus membrane to a chart showing five color categories with "1" being not anemic and "5" representing severely anemic. This is a practical, low-cost method that farmers can use to make deworming and breeding decisions.

Breeding for genetic resistance or resilience is an alternative method of control to reduce parasite affects to livestock. Resistance is a host's ability to initiate and maintain immune response to suppress establishment or eliminate parasites (Woolaston and Baker, 1996). Resilience is the ability of a host to remain healthy and productive while parasitized (Miller et al, 2006). Both resistance and resilience are attributed to the inheritance of genes that result from the expression of immunity from the parental host (Burke, 2019). Selection of sheep breeding stock with resistance to *H. contortus, Nematodirus,* and *Fasciola hepatica* is becoming a popular and effective method for control of these parasites (McManus et al, 2014).

Prevention for gastropod-borne parasites, such as sheep/goat lung worm (*Muellerius capillaris*) liver flukes (*Fasciola* spp.), and meningeal worm (*P. tenuis*) has not been widely explored. Habitat modification, include altering natural habitats (e.g. installing water drainage,

picking up debris), introducing new habitat disturbance (e.g. mowing, raking, rototilling), and adding obstructions (e.g. toxic plants, concrete barriers, salt), or avoiding livestock grazing in wet, snail-abundant areas, may reduce risk. Additionally, intensive land use practices, such as grazing, have been correlated with a decline of snail abundance and simplification of community structure in grassland environments (Whener et al, 2021).

Parasite-related health issues cause economic and management consequences for farmers, wildlife managers, and public health officials. Gaining knowledge of the epidemiology, ecology, and biology of gastropod-borne parasites of livestock is crucial to inform control strategies, to improve animal health, and to lessen the economic burden of disease.

CHAPTER 2. IDENTIFICATION OF PARELAPHOSTRONGYLUS TENUIS ON MAINE SMALL RUMINANT FARMS

2.2 INTRODUCTION

Several parasites of small ruminants use gastropods as intermediate hosts for stages of growth. Among these include, *Parelaphostrongylus tenuis*, a species which reproduces in its natural host, the white-tailed deer (WTD; *Odocoileus virginianus*), and can cause morbidity and mortality in aberrant hosts (dead-end), such as other wild cervids and livestock. This nematode species cannot reproduce within dead-end livestock hosts, thus causing diagnostic challenges for farmers and veterinarians. Sheep lung worm (*Muellerius capillaris*) and liver flukes (*Fasciola* spp.) also use gastropods as natural reservoirs, often over-wintering in the gastropod's tissue (Williams, 1942; Jones et al, 2015). Identification of these gastropod-borne parasites in small ruminant pastures can help inform farmers of the risk level to their livestock.

Previous studies identifying larval *Parelaphostrongylus* species used varied methods. Challenges remain in discerning *P. tenuis* from its close relative, *Parelaphostrongylus andersoni* (*P. andersoni*), a muscle worm that shares a similar life history and has been known to affect the same wild cervid hosts, namely moose (*Alces alces*) and caribou (*Rangifer tarandus*) (Lankester and Haura, 1989; Verocai et al., 2020). *P. andersoni* was found in the Southeastern and Great Lakes regions of the United States, as well as Northcentral and Eastern Canada, but the full range of the worm is poorly documented (O'Leary et.al, 2019; Prestwood we al., 1974; Pursglove, 1977; Lankester and Hauta, 1989). Efforts to locate *P. andersoni* in Maine have been few with no documentation of the worm within Maine WTD or gastropod hosts (Bogaczyk, 1992; Gleich et al., 1977). Given these studies are outdated, and that climate-driven migrations of other

parasites have been observed, it is not unreasonable to suppose the presence of this species could now be in Maine.

Despite many years of efforts to differentiate larval *Parelaphostrongylus* species, inconclusive results continue to occur frequently. Morphologic measurements were traditionally used in many foundational studies of *P. tenuis*. Ballantyne and Samuel found that measurements of *P. tenuis*, *P. andersoni*, and *P. odocoilei* were similar, though characteristics of the posterior end may help discern species (Ballantyne and Samuel, 1984). Early genetic studies have successfully identified *P. tenuis* by amplifying various gene regions such as the second internal transcribed spacer (ITS-2) of ribosomal DNA (Gajadhar et al., 2000; Jenkins et al, 2006), mitochondrial cytochrome c oxidase I (CO1) (Asmundsson et al., 2008; Eggert et al., 2021), 18S (Carreno and Nadler. 2003), and 28S with large subunit ribosomal ribonucleic acid (LSU) (Carreno and Nadler. 2003). Pidwerbesky et al. found cooccurring *P. andersoni* and *P. tenuis* larvae in WTD fecal pellets in northern Manitoba, a new recorded location for the muscle worm, using ITS2 and CO1 gene regions (Pidwerbesky et al., 2023). Most studies design primers to identify *P. andersoni* larvae based on local adult references, however few sequences are available for reference on GenBank, especially of mitochondrial CO1. Additionally, there has been new developments in antemortem serological diagnostic tests to identify *P. tenuis* in moose sera and efforts to gain definitive diagnostics of *P. andersoni* are underway by researchers, though this development has not been established for small-ruminant or livestock diagnosis (Richards et al., 2023). Advancement of genetic technologies allowed us to use novel methods for molecular identification of *P. tenuis* and other nematodes within gastropod collections.

This study explores the naturally occurring parasites found within snails and slugs collected from Maine farms. The objectives were to genetically confirm *P. tenuis* larvae in

gastropods collected from small ruminant farms to document other helminths utilizing gastropods as reservoirs or intermediate hosts, and to investigate the possibility *P. andersoni* presence in Maine. Genetic barcoding and nanopore sequencing were used in this study to target the aforementioned genetic regions, with a goal to compile a comprehensive genome of *P. tenuis.* To determine the species identity of larvae present in land snails, we used genetic analysis of the CO1 gene, ITS2 gene, 18S gene, and 28S/LSU gene regions as well as morphologic characteristics. We predicted that morphologically identified larvae suspected of being *P. tenuis* would be confirmed genetically and we did not anticipate finding *P. andersoni*. This study is the first to examine naturally occurring *P. tenuis* in terrestrial gastropods on small ruminant pastures. This is also the first attempt at using novel sequencing methods using a four-primer multiplex PCR protocol specifically designed for *P. tenuis*. This research provides material to calculate transmission risk to small ruminants and will contribute needed genetic information to be accessed in the public GenBank database.

2.2 METHODS

2.2.1 Sample collection and processing

Gastropod processing: Gastropod hosts were collected from six Maine small ruminant farms (Figure 2) from May- September of 2021 and 2022. WTD use in, or around, all pastures were observed throughout the study. A total of 5519 gastropods were collected. All gastropod samples were examined individually, except for small (≤6mm) snails, which were pooled into groups of like sizes (shell length) and contained to 1cm² of space within the test tube (i.e., three 4-5mm snails; four to five 3mm snails; five to seven 1-2mm snails). Snails and slugs were sliced or crushed, 3ml of 0.6% pepsin- 0.7% hydrochloric acid solution poured over, then incubated overnight at 37°C to digest the tissue. Tubes with digested snails were pipetted into six-

chambered well plates with each tube flushed with water and pipetted again into the same, respective cell. Empty tubes were held before washing so that if larvae were found, another flush of water would be added and examined to determine if any larvae remained. Examinations of gridded well plates were performed on Zeiss and Olympus inverted microscopes with 10x, 20x, and 40x magnification.

Larval collection: Parasite larvae were noted as third-stage (i.e., alive, or dead and intact cuticle/ sheath) or second-stage (i.e., dead and tissue degradation). Third-stage larvae were imaged to include full length photos at 10x, 20x, or 40x magnification, and anterior (head to base of esophagus) and posterior (esophagus and anus to tip of tail) positioning at 20x or 40x magnification. Images of larvae contained a scale (μm to mm) respective to magnification and stored with file names containing magnification, snail ID, larval ID, and whole body, anterior, or posterior position indication. Third-stage larvae were collected and stored in 3-5ml nuclease-free water, then frozen at -20°C. Identical larvae within the same sample were pooled into the same tube (~3-4 pooled larvae per tube). A total of 195 samples from 55 gastropods were recovered and stored for DNA analysis.

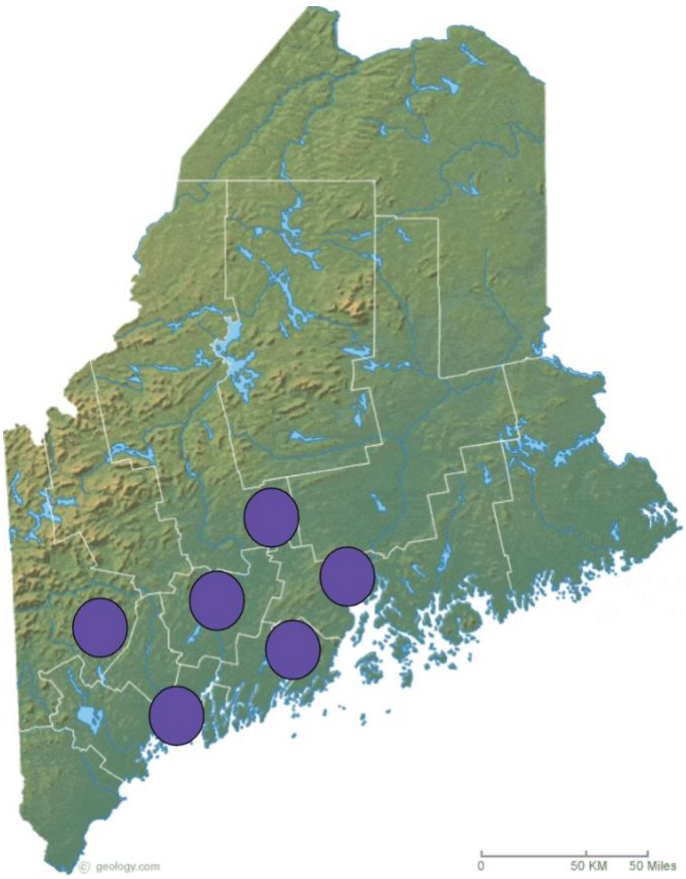

Figure 2. Locations of gastropod collections from May- September 2021 and 2022.

2.2.2 Morphological identification of parasites

Length of imaged nematodes (μm) were measured using ImageJ software (Schneider et al., 2012). Posterior morphological characteristics were key to determining *P. tenuis* larvae, as they have a distinct "hump", or double hump, prior to the short tip of tail (Figure 3). Larvae with this feature, and of 800-1250 μm in length, were labeled as *P. tenuis*. Larvae with elongated humped posteriors and/or with dorsal spines and measured lengths of 400-700 μm were labeled as *M. capillaris* (Figure 2). All other third-stage larvae with no humped tail were documented as "unknown."

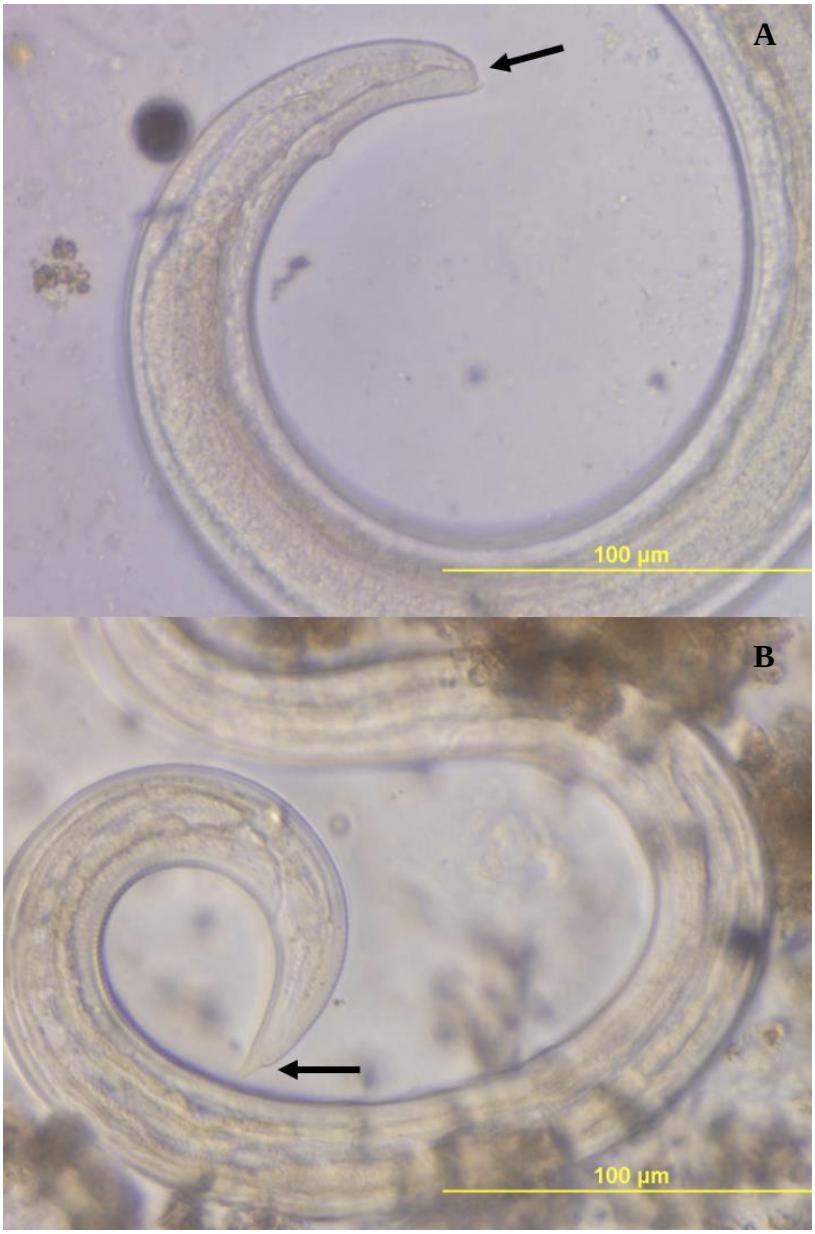

Figure 3. Posterior differences between *P. tenuis* and *M. capillaris.* A) *P. tenuis* L3 with a double-humped tail and short tip. B) *M. capillaris* L3 tail with an elongated tip. Black arrows point to tail hump.

2.2.3 DNA extraction

Total genomic DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany). Adult *P. tenuis*, obtained from a naturally deceased WTD crania, post death ~24 hours, to use as a positive control, were sliced and processed in a bead tube for extraction preparation. Stage-one *M. capillaris* larvae from infected sheep (acquired during preliminary study from University of Maine Witter Farm sheep flock) were used as a positive control for that species. Negative controls were used with distilled water.

2.2.4 Library preparation

Four primer pairs were selected for identification of universal nematode (18S and ITS2) or *P. tenuis*-specific with potential to differentiate between *P. andersoni* (CO1 and 28S/LSU) and were tested individually before use in the multiplex. We amplified ~800-950 bp of the CO1 mitochondrial gene using a designed forward primer CO1–10F (5′-TGGTTTGTGGTCTGGATGGT-3′) and a reverse primer CO1_848R (5′-CCGCAGTAAAATAAGCTCGAGAATC3′). We also amplified ~600 bp of the ITS-2 region of the nuclear ribosomal DNA using the forward primer ITS2-F (5′-ACGTCTGGTTCAGGGTTGTT-3′) and the reverse primer ITS2-R (5′- TTAGTTTCTTTTCCTCCGCT-3′). Another amplification of ~1700bp was the 18S region with forward primer 18S_1F (5’-CGCTATATGCTCAGTTAAAAGATTAAGC-3’) and reverse primer 18S1765R (5’-TGATCCTTCTGCAGGTTCACCTAC-3’). We included the rRNA 28S region with ~3000bp, forward primer Pt28S_115F (5’-CGCTGAATCTTTCGATGTTAAATCG-3’) and reverse LSU_3180R (5’- CTTCGCAATGATAGGAAGAGCC-3’). The NextGen PCR protocol (PCR #1) included a 20 µl reaction that consisted of 2.8µl of distilled water, 10µl repliQA toughmix (Quantabio,

Beverly, MA), 0.4µl of each forward and reverse primer pair (x3), and 6µl of DNA sample. The amplification was performed in a thermocycler with the following conditions: 98 ◦C for 15 seconds, 14 cycles of 98 ◦C for 15 s, 68 ◦C for 20 s, 68 ◦C for 40 s and held at 4 ◦C. We used electrophoresis to visually confirm bands in a 2% agarose gel. We purified PCR products with a Qiagen QIAquick PCR purification kit and DNA yield was quantified on a QFX fluorometer (DeNovix). Library dilution of some sample was necessary prior to PCR2 and nanotag binding. Primer pair sequences with nanotags can be found in the Appendix (Table A2). We diluted samples according to an fmol value of < 0.1 and following band brightness levels: bright= 1:100, moderate = 1:10, faint or no band= no dilution. For ligation in preparation for PCR2, 30µl of the sample DNA from PCR1, 12.5µl ligation buffer, 5µl of NEBNext Quick T4 DNA Ligase, and 2.5µl of ligation adapter were combined for a 50µl reaction. The reaction was suspended in AMPure XP beads and incubated on a Hula mixer for 10 minutes at room temperature. This was spun down and pelleted on a magnet and the supernatant was removed. Beads were washed with 125µl of short fragment buffer, pelleted and liquid removed. The pellet was resuspended in 7µl elution buffer and incubated at 37 ◦C for 10 minutes. The 7µl of eluate containing the DNA library was removed and 1µl was quantified by using a fluorometer.

2.2.5 Sequencing and filtering

We performed high-throughput sequencing of all the target gene regions for the purpose of parasite taxonomic identification using MinION (Oxford Nanopore) technology after the barcoded DNA library went through adapter ligation and clean up. A new flongle flow cell (MinION R10.4.1) per 48 samples was read for number of viable cells prior to library loading. The flow cell was flushed and loaded with a sequence mix of 5µl DNA library, 15µl sequencing buffer, and 10µl library beads. Sequencing was run overnight on MinION real time analysis

platform. The sequence pipeline included separating FastQ files into each primer. Samples with at least 28 reads were targeted for analysis. Contigs were constructed and aligned in Geneious Prime and compared to GenBank accessions using BLAST (Benson et al., 2013) with e values of 0.00 and 98% identity.

2.3 RESULTS

Molecular analyses using nested multiplex PCR and MinION high-throughput was successful in identifying *P. tenuis* and other nematodes. A total of 195 samples were extracted from 55 gastropods; 125 samples from 45 gastropods successfully sequenced. Six species of nematodes were identified (Figure 4 and Figure 6): *P. tenuis* (n=105), *Caenorhabditis sp*. (n=5), *Oswaldocruzia filiformis* (n=4), *M. capillaris* (n=12), *Crenosoma sp*. (n=10), and *Uncinaria stenocephala* (n=1). Three gastropods had co-infections with multiple larval species; one snail contained *P. tenuis* (n=16) and *M. capillaris* (n=7), another had *P. tenuis* (n=11) and *Crenosoma sp.* (n=9), and the third had *P. tenuis* (n=15) and *Crenosoma sp.* (n=1). No *P. andersoni* larvae were identified. A total of 70 samples failed due to overabundant quantities of bacterial or snail DNA. By incidental finding within the 18S loci from 10 sequenced larval samples, eight snails were identified as *Succinea putris*.

Primer success varied per species (Table 1). The 28S + LSU primer pair failed completely, though the remaining primers did work. *P. tenuis* was identified by ITS2 (n=38, 36%), 18S (n=7, 6.7%) and CO1 (n=105, 100%). *M. capillaris* was identified by ITS2 (n=12, 100%) and by 18S (n=3, 25%). *Caenorhabditis* was identified by ITS2 (n=5, 100%). *O. filiformis* was identified by ITS2 (n=4, 100%) and with 18S (n=2, 50%). *Crenosoma sp*. was identified by ITS2 (n=10, 100%). The single *U. stenocephala* sample was confirmed with both ITS2 and 18S.

Table 1. Genetically identified larval helminths with successful primers.

Species	Total (n)	Primer ID Success (n)	
P. tenuis	105	ITS2	38
		CO1	105
		18S	7
		28S/ LSU	0
M. capillaris	12	ITS2	12
		CO1	0
		18S	3
		28S/ LSU	0
Caenorhabditis sp.	5	ITS2	5
		CO1	0
		18S	0
		28S/ LSU	0
Crenosoma sp	10	ITS2	10
		CO1	0
		18S	0
		28S/ LSU	0
O. filiformis	4	ITS2	4
		CO1	0
		18S	2
		28S/ LSU	0
U. stenocephala	1	ITS2	1
		CO1	0
		18S	1
		28S/ LSU	0

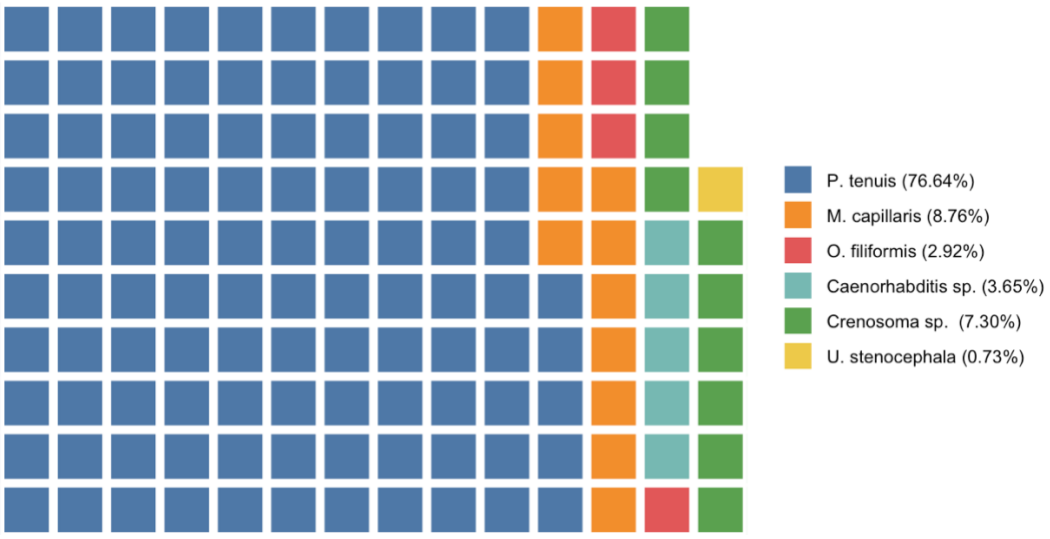

Figure 4. Waffle plot of genetically identified helminth species (N=137) from 125 samples retrieved from 45 gastropods.

Morphological identification of imaged larval samples included body length measurements and observation of humped tail. In total, 208 digested larval helminths from 88 gastropods were measured and identified (Figure 5). Two trematodes were dissected directly from two individual snails and were measured. Images of larvae that were too degraded were not recorded, though some clearly looked to be developing stage-two *P. tenuis* larvae. *P. tenuis* length ranged from 690µm- 1328µm and showed slight tail variation among the individuals. *M. capillaris* length ranged from 454 µm-735µm. The unknown worms ranged from 196µm to 1369µm. The trematodes recovered from snail were 7.2mm and 5.8mm and identified as brown-banded broodsacs (*Leucochloridium variae*; Figure 7). There were four samples with discrepancies between morphological and genetic identification. These were genetically identified as *P. tenuis*, however three were morphologically identified as *M. capillaris*, one was unknown. The morphological identification for the four samples was reflected in Figure 4. Descriptive statistics for measured larvae can be found in the Appendix (Table A.1).

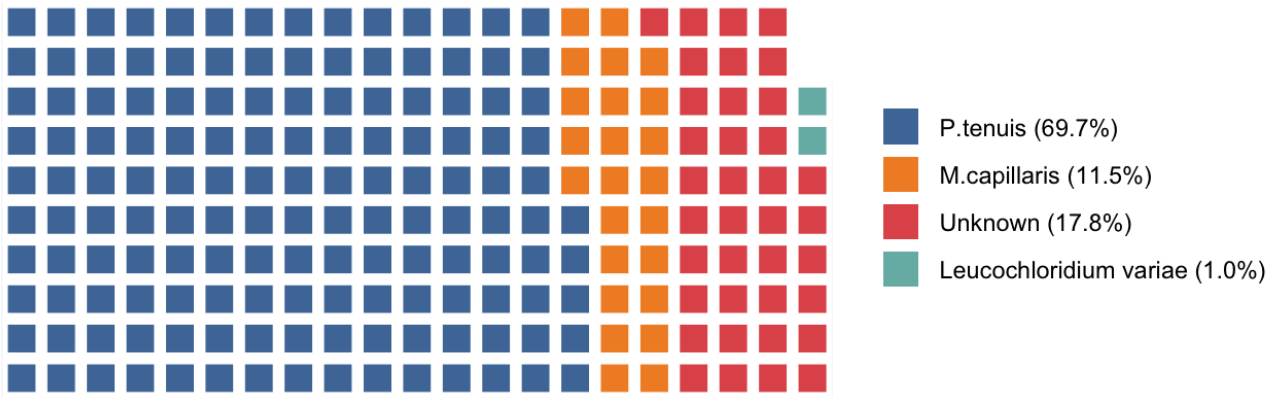

Figure 5. Waffle plot of morphologically identified nematode species (N=208).

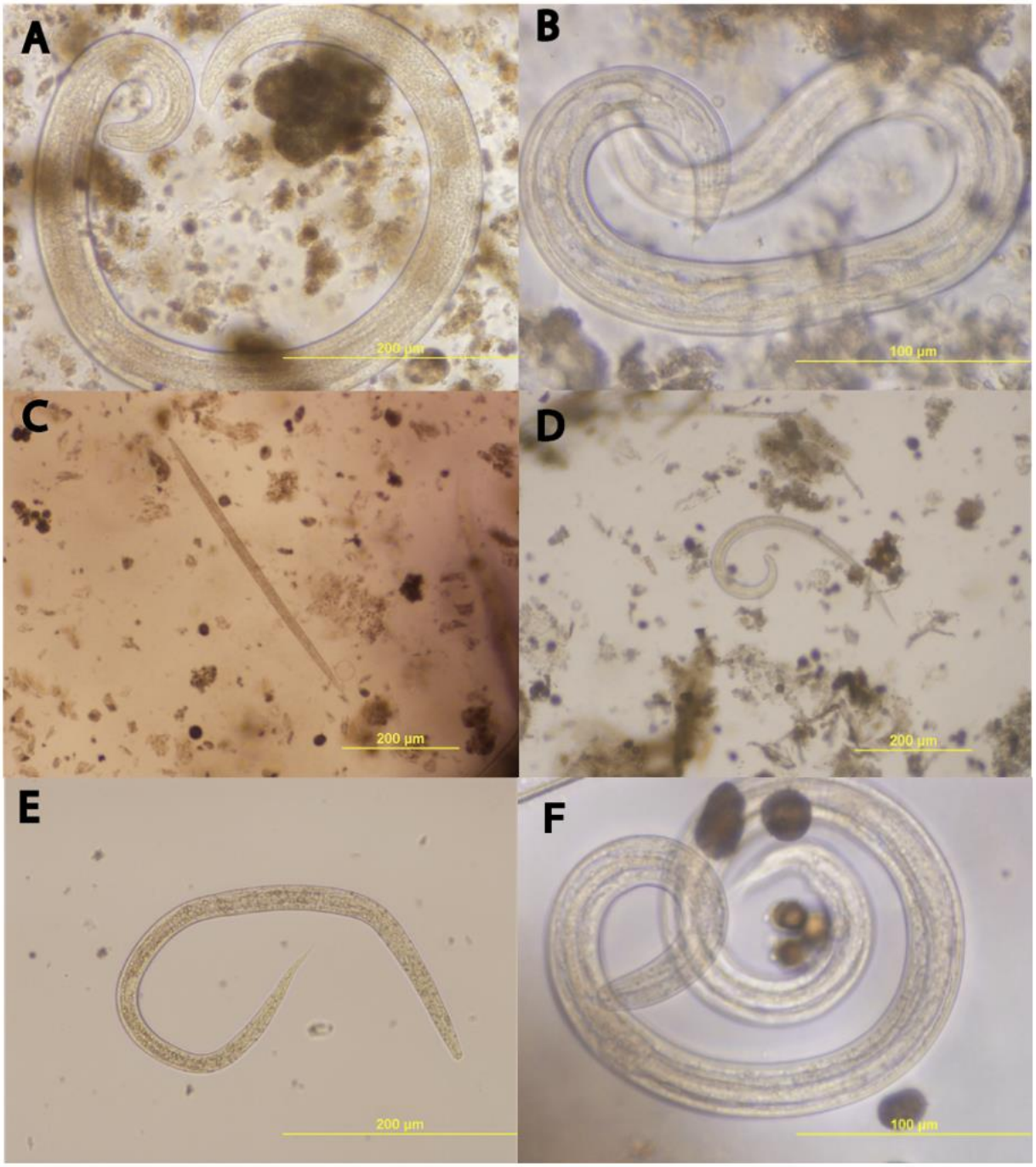

Figure 6. Images of collected larvae. A) *P. tenuis*, 20x; B) *M. capillaris*, 40x; C) *Caenorhabditis sp.*, 10x; D) *U. stenocephala*, 10x; E) *O. filiformis* (20x). F) *Crenosoma sp.*, 40x.

Figure 7. Brown-banded broodsac (*Leucochloridium variae*) in the ocular tentacle of a Succineidae snail.

2.4 DISCUSSION

In our examination of terrestrial snails and slugs on grazing systems shared by small ruminant and WTD, we found that *P. tenuis* had a higher abundance than any other helminth. One other parasite of small ruminants, *M. capillaris*, was also confirmed genetically. No *P. andersoni* larvae were found. Many of the "unknown" nematodes from our morphologic analysis were identified genetically as environmental nematodes, or those with canid definitive hosts, though these pose no known risk to small ruminants.

In our genetic analysis, 125 of the 195 larval samples amplified and sequenced. *P. tenuis* was found in 84% (n=105) of the samples. *M. capillaris* was found in 9.6% of the samples. In one instance, both *P. tenuis* and *M. capillaris* were documented as co-occurring in one snail host (Appendix Table A3; snail ID 4779). One gastropod carried living *Uncinaria stenocephala* (0.8%), a common hookworm of canines such as red foxes (*Vulpes vulpes*), coyotes (*Canis latrans*), and domestic dogs (Wapenarr et al., 2013). *U. stenocephala* are not known to use intermediate hosts, though it can use small mammals as paratenic hosts. Four snails carried

Oswaldocruzia filiformis (3.2%) which is a generalist species with a direct lifecycle frequently found in several amphibian and reptile species (Kirillova et al., 2020). Given the overlap of snails and amphibians in wet habitats, this occurrence is not surprising. Five snails contained larvae identified to the genus *Caenorhabditis* (4%). Species within the genus *Caenorhabditis* are typically generalist species that can be found free living in soils and compost. They have also been observed on and within isopods, insects and land snails though they do not parasitize the hosts, rather use them as transportation to food sources (Li et al, 2014). *Crenosoma sp*. was found within ten samples (8%) from two snails which were both also co-infected with *P. tenuis* (Appendix Table A3; snail IDs 4875 & 4886). Nematodes of the genus *Crenosoma* are lungworms that are commonly found in wild canids and other mesocarnivores, though infections within domestic dogs has been reported (Pohly et al., 2022; Stockdale et al., 1974; Meyer and Chitwood, 1951). The lungworm uses gastropods as intermediate hosts to develop into infective third-stage larvae and are endemic in the Northeast (Stockdale et al, 1974; Shaw et al.,1996). Two brown-banded broodsacs, *L. variae*, were recovered from two snails. These trematodes exclusively parasitize ambersnails of the family Succineidae and cause a pulsating display within the snail's ocular tentacles to attract birds, the definitive hosts (Ohari et al.,2019). The parasite is also called "zombie worm" as it influences the amber snail to climb to the top of vegetation to be more likely predated on by birds. As expected, no *P. andersoni* was identified. Having no *P. andersoni* positive control and availability of only a few Genbank references to access were limiting factors in determining presence of this species.

Amplification success of selected genetic regions varied. ITS2 universally captured all species. CO1 was the most successful in identifying *P. tenuis*. The genetic region of 18S was not very successful in identifying *P. tenuis*, though it was able to identify *M. capillaris*. In our

individual primer testing preliminary runs, 28S/ LSU was successful. However, when combined with the other primer sets in the multiplex, it failed, which was likely due to the temperature differences from the other primer sets. The purpose of the multiplex was to target *P. tenuis* using species-specific genomic code, and for unknown nematodes using universal primers. The ITS2, CO1, and 18S combination was successful in identifying a range of nematodes.

Future directions:

Genetic analysis of larvae using a nested multiplex PCR protocol and the Nanopore sequencing pipeline allows for more robust genomic coverage than other methods. More work to strengthen this method and adding other genetic regions, particularly using 28S/ LSU with ~ 3000 base pairs, should be done to encompass a wide range of lengths which could create a near-whole genome. A whole genome sequence study of *P. tenuis* could help future researchers to identifying this species in comparison with close relatives of the worm. Results from the genetic component of this chapter will be accessible through the GenBank database in the future. Additionally, a phylogenetic comparison with *P. tenuis* larvae from Maine with Northeast and North American larvae could help document similarities and and relationships among species. We attempted to compare the phylogeny of *P. tenuis* across our sample sites, but there was little to no variation. Our findings of a snail with cooccurring parasites leads to questions on the effects of *P. tenuis* and *M. capillaris* on parasite (each other), gastropod, and small ruminant host fitness.

The exploration of different helminths within snails and slugs from pastures revealed that *P. tenuis* is the most abundant parasite. These findings will inform risk of *P. tenuis* on small ruminant farms, which is discussed in the next chapter. Understanding the risk of these parasites is essential when planning management strategies to limit small ruminant exposure.

CHAPTER 3. RISK FACTORS OF PARELAPHOSTRONGYLUS TENUIS TRANSMISSION TO SMALL RUMINANT LIVESTOCK

3.1 INTRODUCTION

Parelaphostrongylus tenuis, also known as meningeal worm, is a nematode that reproduces in white-tailed deer (WTD; *Odocoileus virginianus*) and can cause debilitating neurological damage or mortality in aberrant hosts, such as wild cervids and livestock. Domestic animals such as sheep (*Ovis aries*; Jortner et al, 1985), goats (*Capra hircus*; Guthery, 1979), llamas and alpacas (*Lama glama* and *Lama pacos*; Brown, 1978; Foreyt et al., 1991), horses (*Equus caballus*) and cattle (*Bos taurus;* Duncan and Patton, 1988) grazing on pastures with WTD and gastropod presence may face risk of spillover of the worm (Pybus et al, 1996; Guthery et al, 1979; Ismail et al, 2011; Mittelman et al, 2017; Duncan et al, 1998). Small ruminants, collectively encompassing sheep, goats, llamas, and alpacas, are at increased risk of meningeal worm. Under certain circumstances, the parasite can cause high rates of mortality at the herd/ flock level if left untreated (Guthry et al, 1979; Alden et al., 1975; Keane et al., 2022).

In the definitive host (WTD), the complex life cycle of *P. tenuis* starts with a gravid female adult worm within the WTD's cranial venous sinuses and subdural space; eggs are released into the venous blood (Anderson, 1963). The eggs hatch within the lung capillaries, and stage-one larvae (L1) migrate into the deer's bronchioles and move up the respiratory tract until they reach the pharynx, where they are coughed up, swallowed, and then thus enter the digestive tract. These L1 are then expelled within the mucus layer around the WTD's feces and may be ingested or absorbed by snails or slugs. While in the intermediate gastropod host, these larvae develop into second- and infective third-stage (L3). The cycle starts again when a WTD ingests a snail or slug that harbors an infective stage larva while browsing. The ingested larva travel

within the WTD gastrointestinal tract, then migrate along the spinal nerves, then along the spinal cord as they mature, finally entering the subdural spaces surrounding the brain.

In contrast, if a small ruminant host ingests an infected gastropod, the life cycle of the meningeal cannot be completed, therefore these animals are referred to as "dead-end" hosts (Figure 8). The migration of the L3 larvae can cause cerebrospinal nematodiasis in which animals may suffer from neurological effects such as ataxia, stiffness, circling, blindness, head-tilt, and loss of fear of humans, or death (Anderson, 2000). Incubation period in aberrant hosts is thought to be around 45-53 days (Rickard et al., 1994). There is evidence that some aberrant hosts may develop an immune response to *P. tenuis* if infected in low doses or if treated with anthelmintics at the onset of symptoms and reinfected up to one year later (Ogunremi et al., 2002; Purdy et al., 2012). The lack of antemortem tests results in diagnostic challenges for confirming incidences, though the development of a serological assay for *P. tenuis* in moose shows promise for the advancement of veterinary diagnostics for the worm (Richards et al., 2023). Definitive diagnosis is primarily confirmed through response to treatment or gross necropsy.

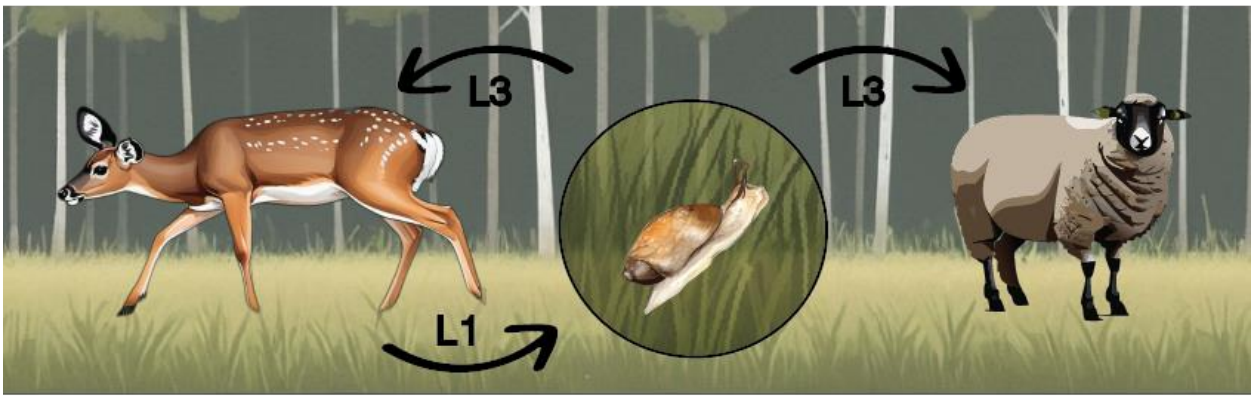

Figure 8. Transmission of *P. tenuis* to aberrant hosts. Stage one (L1) larvae is expelled in the feces from the white-tailed deer definitive host, gastropods act as intermediate hosts and the larvae grows to infective stage three (L3). Infected snails with L3 are ingested by browsing deer or in aberrant "dead end" hosts, such as sheep. Graphics provided by openart.ai.

Behavior of WTD definitive hosts, and the prevalence of meningeal worm affects the risk of intake by livestock aberrant hosts. Karns (1967) supposed that parasite prevalence is deer density dependent; Behrend and Witter's (1968) research of meningeal worm prevalence in Maine deer supports the density-dependent theory, finding an average of 84% worm prevalence and up to 100% in high density areas (density informed by road kill and hunting statistics- no exact https://www.facebook.com/share/p/PzJCr2XR9i1BmK6R/ density values given). In contrast, Gilbert (1973) found *P. tenuis* prevalence in WTD from Maine ranged from 59% in the highest deer-density areas (10-15 deer/ square mile) to 81% in low density areas (3-5 deer/ square mile). Anderson (1963) found worm presence in deer is higher in the summer than in the winter.

Meningeal worm-induced effects to livestock can be attributed to larval intensity, or load ingested by the animal, as suggested by several experimental studies that administered varying

larval challenges to animals (Anderson & Strelive, 1966; Pybus et al, 1996; Ismail et al, 2011; Rickard et al., 1994; Foreyt et al., 1991). These studies showed that small ruminant species have differing thresholds of *P. tenuis* challenges, with camelids (llamas and alpacas) being the most sensitive to low larval load (~5 larvae). Incubation period is also affected by *P. tenuis* larval load; higher larval loads result in faster onset of symptoms caused by the worm (Rickard et al., 1994, Foreyt et al., 1991). Within the host, a small number of larvae are sometimes killed, presumably from immune response, before reaching the central nervous system, suggesting larger doses may be more successful at causing illness (Pybus et al, 1996; Purdy et al., 2012).

Several species of gastropods can act as an intermediate host for *P. tenuis* and other small ruminant parasites. In 1968, Anderson and Lankester examined almost 10,000 gastropods for meningeal worm. They classified twelve gastropods as intermediate hosts for *P.tenuis*, with about 4% being infected (Lankester & Anderson, 1968). In Maine, Gleich et al. (1977) examined forest dwelling gastropods and found that 4%-19% of snails and slugs carried a range of nematodes, but *P. tenuis* was only found in *Pallifera* slugs. Sheep lung worm (*Muellerius capillaris*) and liver flukes (*Fasciola* spp.) also use gastropods as natural reservoirs, often over-wintering in the tissue (Williams, 1942; Jones et al, 2015).

Climatic conditions supporting host survival and larval uptake have been extensively studied in wild habitats. For example, Lankester (2018) found that mild winters and early springs increase deer survival, resulting in excess production of first stage larvae. Stage-one larvae within WTD fecal pellets, and growing larvae within hibernating gastropods can overwinter and survive freezing temperatures (Lankester, 2011; Jenkins et al., 2006). This, in combination with active and abundant gastropods in their spring breeding cycle, results in increased potential of

larval ingestion by mammalian hosts. In contrast, arid regions (with low precipitation and humidity and high ambient temperatures) have lower risk of *P. tenuis* exposure since this climate does not support terrestrial gastropods, nor allows L1 larvae to stay viable in fecal pellets for long (Anderson, 1972; Slomke et al. 1995; Jaques et al., 2015).

Environmental factors such as vegetation type, soil moisture or content, presence of a natural water source, and topography can influence risk level of gastropod-borne parasite transmission. In forested settings, deer are more likely to be reinfected by meningeal worm in habitats with less upland deciduous forests and more coniferous shrubby areas, and in areas that were cool, wet and shaded (Vanderwaal et al., 2015; Wasel et al., 2003; Cyr et al. 2014). Anderson (1975) found that low, damp forests contained more gastropods with higher meningeal worm prevalence than in high dry forests. He also suggested that grassy fields are important area for transmission to deer because, though gastropod numbers were low, the proportion infected was high. Many gastropods favor soils with high moisture and high calcium content (for shell building), or on calcium-poor soils with vegetation that have concentrated calcium in its leaves (Martin, 2000; Martin and Sommer, 2003). These factors have not been well documented for meningeal worm transmission on agricultural pastures.

Farming systems that promote habitat for beneficial invertebrates, including gastropods, and with WTD presence, may face risk of *P. tenuis* transmission to livestock. This project seeks to examine the factors influencing risk of gastropod-induced parasite transmission to small ruminants on grazing space. By investigating intermediate host characteristics including snail and slug species, host habitat preference, and *P. tenuis* intensity may be calculated. It is hypothesized that snail- and slug- specific factors, such as gastropod size, influence larval presence and abundance. Also, it is expected that with higher gastropod abundance on the

landscape at specific times of year, more L3 *P. tenuis* will be found than in other times of year, thus posing increased risk to small ruminants based on seasonal differences and host abundance. It is also predicted that infected gastropod presence be observed in aggregations where there are preferred microclimates, rather than dispersed uniformly throughout the pasture. Documenting risk factors, such as gastropod species and pasture location, may inform preventive management strategies for small ruminant managers. The objectives for this study were to determine infective-stage *P. tenuis* transmission risk factors to small ruminants.

3.2 METHODS

3.2.1 Study area

After an online recruitment effort that yielded seven responses, six farms in Maine (Figure A.1) were selected based on project criteria, which included having thirty or more small ruminants (i.e., goats, sheep, alpacas, and/or llamas), reporting an abundant and active deer population, and having observed gastropods on pastures. Two grazing fields of approximately two hectares per farm were chosen randomly, with exception of Farm E where only one field was available for sampling due to land access limitations. These fields were mapped, gridded, and flagged to create 10m x 10m plots. Farm visits occurred in the spring and summers (May-September) of 2021 and 2022 for a total of ten visits per year per farm.

3.2.2 Gastropod collection and processing

On each farm, the study pastures were gridded into 10m x 10m plots and each plot was coded. Sampling sites were selected via stratified random sampling of coded plots to include center (starting 10m away from fence on the inside), outside (0-10m from fence), and verge (i.e., fence line up to 10m to the inside) at four grids per location type (N=12). Within the 12

selected pasture plots, PVC quadrats measuring 45cm x 90cm were randomly placed to mark smaller sampling areas from which gastropod and environmental data were collected. A schematic of a gridded field and quadrat sample photo is found in the Appendix (Figure A.2).

All gastropods within the borders of the quadrat were collected during a maximum search time of ten minutes. Gastropod searches started from the top of vegetation and worked down to the plant's surface roots. Specimens were hand-plucked, counted, field identified to family or genus level, then placed in a Ziplock bag with alike organisms from that sampling quadrat. Bags were stored in refrigeration (2.7°C) for no more than five days until processing.

In the laboratory, the gastropods were examined individually, identified to family, genus, or species, and body and shell size measured. Snails were categorized into groups by shell size: large (14mm to 19mm), medium (9mm to 13mm), and small (8mm and below). Snails were placed in test tubes individually or, if they were 8mm and less, pooled into groups of like species and sizes and placed within a single test tube if the volume was less than one cubic centimeter (e.g., three 4-5mm snails; four to five 3mm snails; five to seven 1-2mm snails). Slugs were identified, length measured, and placed individually in six-chambered well plates (one per well; CellPro™). Single or pooled gastropod samples were sliced with dissecting scissors or crushed using the blunt end of a nickel lab spatula, then 3ml of a 0.6% pepsin- 0.7% hydrochloric acid solution was added. Samples were then incubated overnight at 37°C (Nankervis et al., 2000). Tubes with digested snails were pipetted into six-chambered well plates (one well per tube); each tube was flushed with distilled water and repipetted again into the same, respective well. Empty tubes were held aside before washing so that if larvae were found, another flush of water would be added and examined to determine if any larvae

remained. Examinations of gridded well plates were performed on Zeiss and Olympus inverted microscopes with 10x, 20x, and 40x magnification.

Larval collection: Larvae were collected from digestates and stored in 3-5ml nuclease-free water, then frozen at -20°C. When identical larvae were found within the same sample, larvae were pooled into the same tube (~3-4 pooled larvae per tube). Third-stage *P. tenuis* larvae were identified by their "humped" posterior slope to the tail tip and body length of around 1000μm (Ballantyne and Samuel, 1984) and from genetic analysis as described in Chapter 2 of this dissertation. For statistical analysis, larval load (or intensity) within digested gastropod samples was characterized according to ranges in accordance to the observed effects on small ruminants based on published literature (i.e., low = $\leq$ 5 larvae; moderate dose= 6-11 larvae; high dose= 12+ larvae).

3.2.3 Animal Mortality

As incidence of *P. tenuis* uptake by small ruminants is not quantifiable, documentation of animal response to treatment for presumptive meningeal worm infection, or documentation of death following neurological symptoms of meningeal worm were considered to suggest, but not prove, infections. Necropsy of small ruminants showing neurological symptoms was offered to all farmers in the study.

3.2.4 Statistical Analysis

The study design of two fields per farm (one field on one farm) created issues around independence. The analyses violate independence and requires a statistical approach to evaluate within-field differences; however these single-field case studies are valuable when considering the diverse animal and pasture management strategies found across farms. The effects of month, year, and pasture location on gastropod host and larval abundance, and on host size

were explored using nonparametric regression models. The relationships between these categorical variables on total snail and infected snail (binomial data) abundances were analyzed. Snail shell size effect on larval abundance was also explored using nonparametric regression. All statistical tests were conducted using R v.4.3.0 (R Core Team 2023). Negatively skewed raw data were logarithmic-, cube root-, and square- transformed, though this procedure did not improve normality. Nonparametric data were analyzed with the Kruskal Wallis rank-based one-way linear regression test (Kruskal and Wallis, 1952) to test for significant differences in gastropod and larvae abundance and prevalence by pasture location, month, or year, and then pairwise comparisons using Dunn's test with a Benjamini-Hochberg (BH) p-adjustment for multiple tests. The alpha value for significance was set to 0.05 (5%). Discrete data included abundances, while continuous data included shell lengths. Host characteristic models include *P. tenuis* larval load ~ shell size, *P. tenuis* larval load ~ pasture location, total snail abundance~ pasture location, and infected snail abundance~ pasture location. Pasture location was nominal data. Seasonal effect models include infected snail abundance ~ month + year, infected snail shell size~ month + year, and *P. tenuis* larval load ~ month + year. Outliers in larval load were kept in the analysis, given the importance of high larval loads on animal health. Larval and infected gastropod prevalence were adjusted for variable pool size using Epitools Epidemiological Calculator (Sergeant, 2018).

3.3 RESULTS

3.3.1 Gastropod characteristics

In total, 5519 gastropods were collected and digested. In 2021, 2623 gastropods were collected and in 2022, 2896 gastropods were collected. Gastropods were collected from all farms

in this study: Farm A (n=2907), B (n= 271), C (n=50), D (n=161), E (n=10), and F (n=2120). A total of 981 gastropods were pooled, resulting in 4538 processed samples; this is the number associated with related analysis unless otherwise noted. Five taxonomic groups of gastropods were observed (Figure 9). The family Succineidae, commonly known as amber snails, was the most abundant (n=5373/ 5519 or 97.4%). Others included the slugs, genus *Deroceras* (n=83/5519 1.5%), *Limacus flavus* (n=47/5519 or 0.85%), *Arion fucus* (n=3/5519 or 0.05%), and the snail of genus *Zonitoides* (n=13/5519 or 0.23%)

Succinea snails were the only gastropod family to harbor *P. tenuis*. A total of 51 snails (0.99%) contained third-stage larvae. Of this, 5 infected sample pools, totaling 22 snails (two tubes of two 7mm snails, two tube of four 4mm-5mm snails, and one tube of ten 2mm snails). Each farm had varying quantities of infected snails (Table 2). *P. tenuis*-positive samples were only found on farms A (n=27), B (n=3), and F (n=21). Larval loads of *P. tenuis* within these snails ranged from 1 to 33 larvae (median= 1; mean= 4.58) and a total of 234 individual third-stage larvae were documented. Shell lengths of all Succinea snails in the study ranged from 1mm to 20mm (median= 10mm; mean= 9.33mm). Snail shell size was associated with *P. tenuis* larval load ($H(2)= 16.32$, $p<0.001$) with an effect size of 0.003. Appendix Table A.4 shows shell size larval load ranges. Large snails carried up to 16 larvae (mean= 4.22 ± 5.07), medium snails up to 33 larvae (mean= 5.25 ± 7.80), and small snails carried up to two larvae (mean=1.17 ± 0.41). Larval loads were less in small snails relative to medium snails($p<0.01$) and large snails ($p<0.01$) (Figure 10; Table A.5). There was no difference between large and medium snails in larval intensity ($p=0.08$).

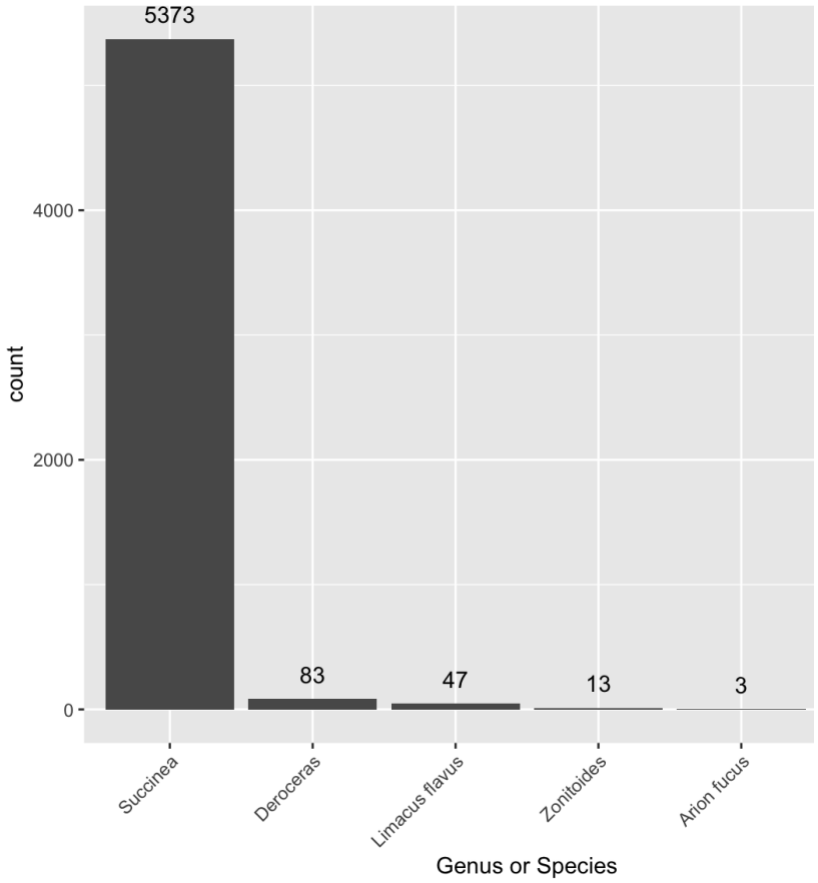

Figure 9. Total gastropods (by family, genus and/or species) collected over two years in pastures selected for study on six Maine farms. Prevalence data includes pooled sample estimates.

Table 2. Numbers of digested gastropod samples and percentage of *P. tenuis*-infected gastropods by farm found on six small ruminant farms in Maine over two years.

Farm ID	**Digested samples (n=)**	***P. tenuis*-infected samples (n=)**	**Gastropods infected (%)**
A	2348	27	1.1 %
B	235	3	1.2%
C	50	0	0%
D	139	0	0%
E	10	0	0%
F	1756	21	0.99%
TOTAL (N=)	4538	51	0.99%

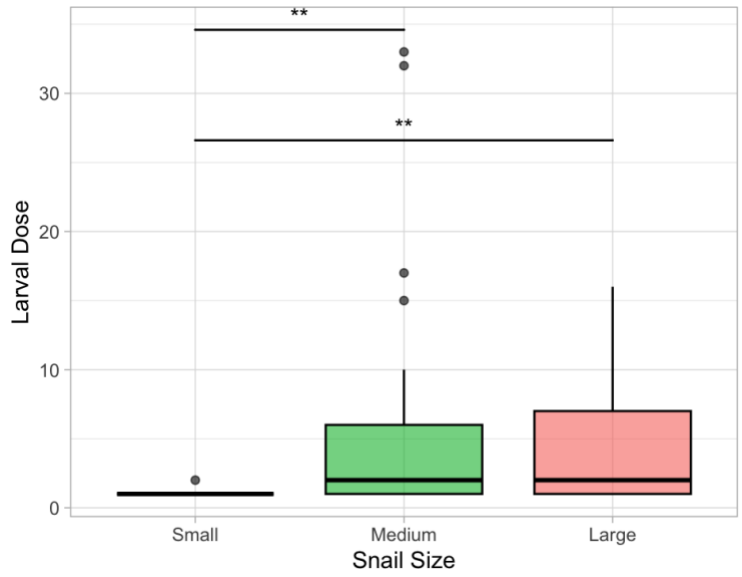

Figure 10. *P. tenuis* larval load by Succinea shell size group. Based on the Dunn pairwise test with BH p-adjustment, larval loads of small snails differed from medium snails ($p<0.01$) and large snails ($p<0.01$). Solid dots indicate outlier values per snail size category.

3.3.2 Farm and Pastures

The location of sampling sites within a pasture (i.e., center, verge, and outside) had varied effects on gastropod and larval populations (Table 3, Figure 11). Succinea snails were found on all pasture location sites with the most snails found in the center (n=2317, 43%), followed by the verge (n=1959, 37%) and outside (n=1097, 20%). Regarding numbers of all gastropods, those found in outside locations were fewer than those in the center and verge ($H(2)= 9.44$, $p<0.001$ and $p<0.01$ respectively). *P. tenuis*-infected snails were collected from locations in the center (n=15), verge (n=24), and outside (n=12). Pasture sampling site had no effect on *P. tenuis* infected snails ($p=0.13$), nor on larval load of *P. tenuis* ($p=0.13$; Figure 12). Tables for

significance and descriptive statistics for all gastropods, infected snails, and larval load per pasture site can be found in the Appendix (Table A.6, Table A.7, Table A.8, Table A.9).

Table 3. Counts of total gastropod, digested Succinea samples and *P. tenuis* per pasture sample location type. Center is central pasture within 10m to the fence or verge boundary, verge is the fence line up to 10m on the inside of the pasture, and outside is the fence line up to 10m on the outside of the pasture.

	Center N (Mean, SD)	Verge N (Mean, SD)	Outside N (Mean, SD)	Total N (Mean, SD)
All gastropods (infected + noninfected)	2385 (1193 ± 33.2)	2034 (1017 ± 257)	1100 (550 ± 97.6)	5519 (1840 ± 664)
P. tenuis-infected Succinea	15 (7.5 ± 2.12)	24 (12 ± 4.24)	12 (6 ± 1.14)	51 (25.5 ± 0.70)
Number of *P. tenuis* larvae	84 (42 ± 28.3)	118 (59 ± 53.7)	32 (16 ± 12.1)	234 (117 ± 83.4)

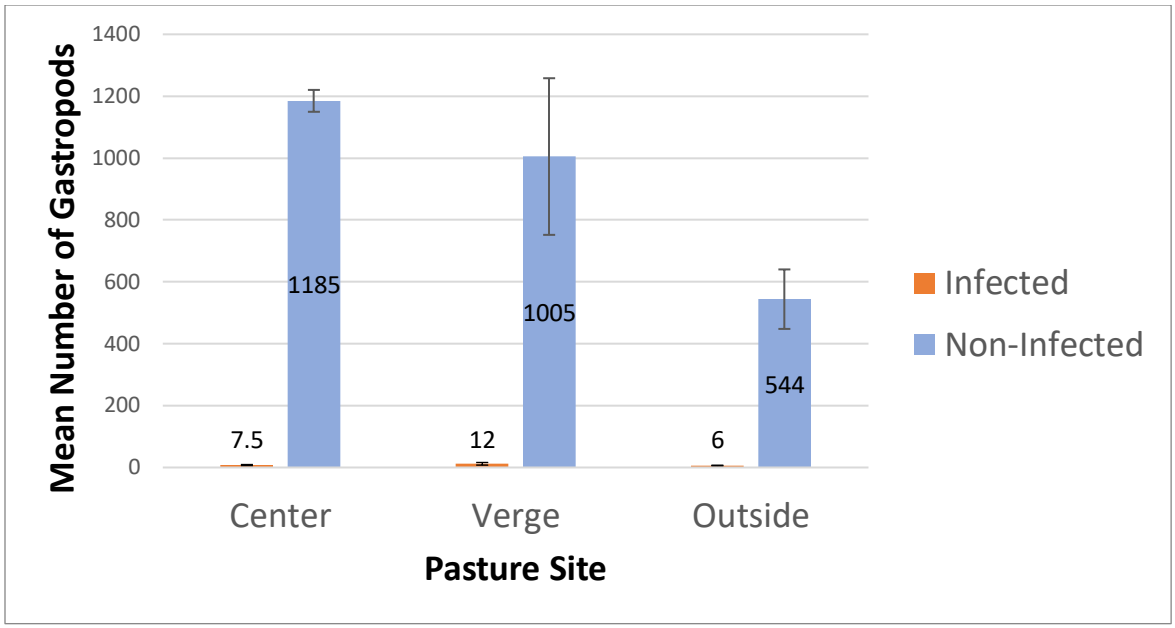

Figure 11. *P. tenuis*-infected snails and non-infected gastropods means and SD per pasture location. Center is central pasture within 10m to the fence or verge boundary, verge is the fence line up to 10m on the inside of the pasture, and outside is the fence line up to 10m on the outside of the pasture.

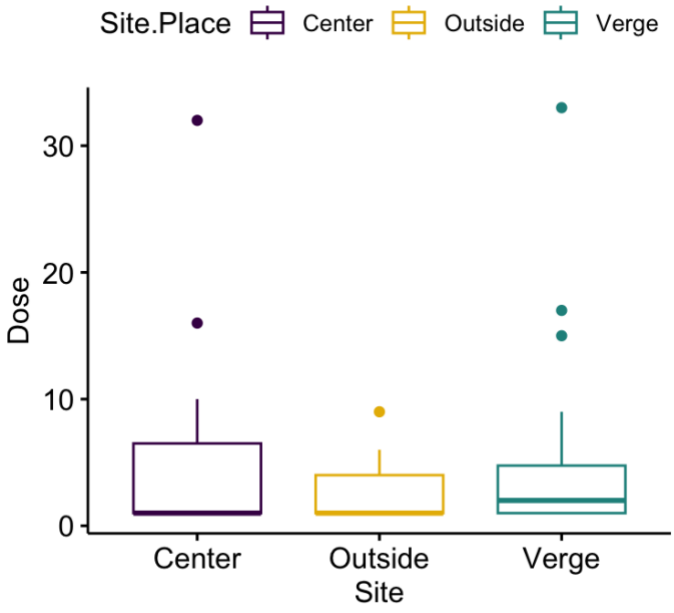

Figure 12. Larval load of *P. tenuis* by sampling site location within a pasture. Center is central pasture within 10m to the fence or verge boundary, verge is the fence line up to 10m on the inside of the pasture, and outside is the fence line up to 10m on the outside of the pasture. Circles indicate outliers.

3.3.3 Seasonal effects

Observations of infected and non-infected gastropods were made from May to September for two years (Figure 13) showed a decline in abundance for all gastropods ($H(5)=29.7$, $p<0.001$), for *P. tenuis*-infected Succinea snails ($H(5)= 38.9$, $p<0.001$), and for larval load ($H(5)=39.1$, $p<0.001$). Infected snail abundance increased significantly from May and July ($p<0.001$), and June and July ($p<0.001$). Larval loads within these snails followed the same pattern (May-July, $p<0.001$ and June-July, $p<0.001$; Figure 14). None of the snail or parasite variables assessed differed by year (all gastropods, $p=0.29$; infected snails, $p=0.83$; larval load, $p=0.84$). Tables of significance and descriptive statistics for estimates of all snails, infected

snails, and larval load by month can be found in the Appendix (Table A.10, Table A.11, Table A.12, Table A.13), and Figure A.3 of infected snail shell range by month.

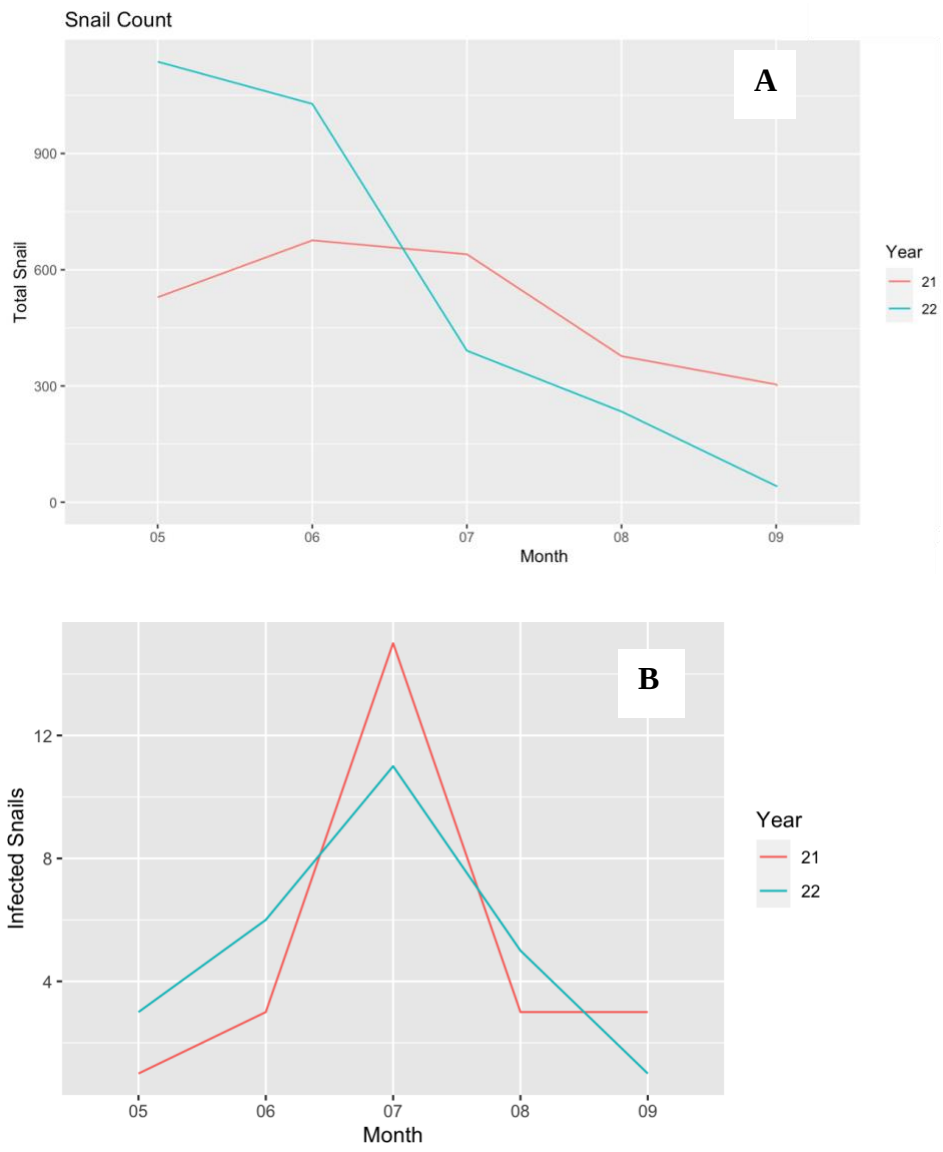

Figure 13. Monthly population trends of Succinea snails and *P. tenuis.* A) Seasonal trends of all Succinea snail populations by months and year. B) Seasonal trends in *P. tenuis*-infected (third-stage) Succinea population by month and year.

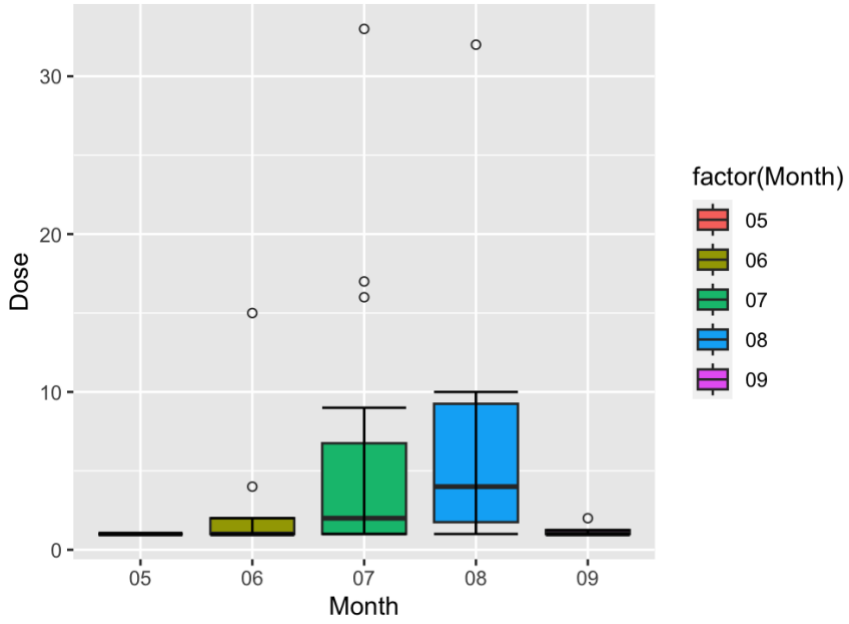

Figure 14. *P. tenuis* larval load within infected gastropods by month. Circles indicate outliers from months.

3.3.4 Animal morbidity & mortality

Several small ruminants over the course of the two years experienced neurological symptoms or death suggestive of *P. tenuis*. In July 2021, Farm D experienced the loss of an adult goat following suggestive symptoms. The animal was not available for necropsy. In January 2022, an adult alpaca and a 10-month-old lamb from farm B died following neurological symptoms. Both were necropsied and *P. tenuis* was ruled out. In April 2022, Farm A lost two, three-week old lambs; *P. tenuis* was ruled out. This same farm had four adult ewes present with meningeal worm-related signs throughout the grazing season in 2022, and all responded to anthelmintic treatment (farmer reported at the end of season). The most likely observation of actual incidence was in August 2022 on Farm F, when an adult ewe became symptomatic, then quickly died following exposure to a site which happened to be a snail collection site at the time. Animals were grazed at the site for 2 days and rotated off four days

prior to gastropod collection, and five days prior to larval examination. Multiple snails in this area contained dangerous loads of 7-33 larvae. The ewe died seven days from the first day of site exposure and was not available for necropsy.

3.4 DISCUSSION

In this study, *P. tenuis* infection risk was predicted to be influenced by high gastropod abundance concentrated in small areas of pasture, by high larval loads within infected gastropods, and to be seasonally dependent. In contrast to the findings of other studies, only one family of gastropods was found to carry meningeal worm: Succineidae, or amber snails. A consistent observation among these farms was that populations of gastropods varied over time. Additionally, each farm's gastropod populations differed greatly, likely due to differing regions of the state, pasture management strategies, and microclimate conditions.

3.4.1 Intermediate host characteristics

Based on observations from snail and slugs collected from small ruminant grazing pastures across Maine, gastropods of the family Succineidae were found to be the primary intermediate host for *P. tenuis*. The homogeneity of intermediate hosts was unexpected and contradicts one of this study's hypotheses that a diversity of species would be found harboring the worm. However, we could only identify to the family level of Succineidae, which includes many species. These results support the findings from other studies suggesting Succinea snails are suitable intermediate hosts for *Parelaphostrongylus* species (Jenkins, et al., 2006; Lankester and Anderson, 1968; Kutz et al., 2000), though they also report the slug, *D. leave* as being the most infected gastropod species and with the high larval intensity (~8%), among other suitable gastropod host species. While these species of slugs were documented in this study, they carried large quantities of *M. capillaris*, or sheep lungworm, but no detectable *P. tenuis*. An attempt was

made to classify Succineidae snails to the genus or species level on several samples with DNA sequencing; *S. putris* was identified, though this can't be confirmed for all amber snails collected. Other studies have reported challenges for morphologically and genetic identification of amber snails; thus, classifying these snails to the family level by morphology was practiced in this study (Jenkins et al., 2006; Perez et al., 2021).

This study found that one in one hundred amber snails on pasture with WTD overlap are infected with *P. tenuis*. In total 51 samples (five pooled; out of 4538 digested samples) contained *P. tenuis* (0.99%). This proportion is lower than the 4% infected *S. ovalis* that Lankester and Anderson (1968) found on Navy Island, Ontario. Most of the gastropods in this study were found on two farms (Farms A and F), with infected snails found on farms A (n=27), F (n=21), and B (n=3). Numerous degraded larvae were observed in digests, though those were not included in this analysis as they could not be confirmed as *P. tenuis*. Because gastropod collections were randomly selected across field sites for each visit as to eliminate removing hosts from the same population, it is possible that the average proportion of infected gastropods could be different if examining the same population/ site throughout a season.

Presence of large and medium snails on grazing space may pose a higher threat of larval infection by livestock. Snail shell length was correlated with larval intensity; large (14mm+) and medium (9-13mm) snails contained relatively high meningeal worm loads (up to 33 larvae, mean= 4.22 and 5.25, respectively). Small snails ($\leq$ 8mm) that were infected contained one-to-two larva. Ingestion of a single snail with a high larval load, or of multiple snails with low-to-moderate numbers of larvae can increase the risk of livestock illness and potential death. In small ruminants, especially camelids, sensitivity to meningeal worm infection is reported to be load-dependent. Experimental doses of five or more larvae can cause neurologic symptoms, and

high doses (above 12 larvae) can expedite symptoms and mortality, which was suspected in one animal death during this study (Rickard et al., 1994). One reason that larger snails contain higher amounts of larvae could be that they are further along in their life cycle and closer to death than smaller snails, thus having lowered immunity. Jenkins et al. (2006) found that disproportionally high numbers of protostrongylid larvae were in sick or dying snails, which were often physically large. They suggested that the larvae were sensitive to the internal environment and immune system of the gastropod host.

3.4.2 Seasonal effects

Over the two years of this study, months significantly affected the numbers of the general population of Succinea snails across all sample locations, the prevalence of *P. tenuis*-infected Succinea, and the larval intensity within infected snails. Succinea snails had high abundance during the spring months (i.e., May and June), then progressively decreased in abundance starting July until September. The number of snails infected with L3 *P. tenuis* showed a different trend from the general population, presumably reflecting the stages of which the worm matured within the host. This trend showed low numbers in May, increasing to maximum abundance by July, and then numbers decreased by August. By August, the two years' infected snail trends differed, with 2021 showing a surge of abundant infected snails, and with 2022 showing a continuation of decline. These patterns on infected gastropod population variability over the course of the grazing season follows a similar monthly pattern to a study by Keane et al. (2022). Their findings over 18-years of postmortem diagnostic examinations of *P. tenuis*-caused mortalities showed seasonality of animal mortality, with October to December showing high mortalities, a decline of deaths until May, a slight surge of cases from May to July, and finally a drop off of cases to August which turns to an abrupt surge of cases going into the autumn. Their

observance of mortality trends from May to July (increase) and July to August (decrease) is mirrored in infected snail abundance trend by the present study shows, though their estimated mortalities were about four times greater from October-December than May-June. It is hypothesized that the May-June mortalities were exclusively caused by consumption of highly abundant overwintered larvae within older, larger dormant snails shortly after emergence, and that the late summer- into fall mortalities were caused by a combination of spring-hatched medium and small sized snails (consumption in May-June, with smaller larval loads resulting in a slower incubation period) as well as dying large snails (consumption in July-August with high larval loads resulting in a quicker incubation period), thus creating the triple-fold spike of mortalities at that time period. *P. tenuis* incubation period in aberrant hosts is still not well understood, but factors such as host age (younger animals) and quantity of infective-stage larva ingested have been attributed to the onset of symptoms ranging from 4-71 Days (Anderson and Strelive, 1969; Dew et al., 1992; Rickard et al., 1994; Purdy et al., 2012).

The life cycle and phenology of Succinea snails on the landscape may influence risk. Succinea are hermaphroditic and typically will self-copulate when the population is stressed (Dillen et al., 2009; Orstan, 2010). This potentially allows for persistence of the snail host population without intervention from negative environmental influences or chemical intervention. Additionally, like most gastropods, Succinea become dormant from November to March or April, and in Northern climates, moves into the soil until spring (Orstan, 2010). Orstan 2010 studied population cycles of *Oxyloma retusum*, a common member of the Succinidea family, in Maryland. He found that snails reached their largest size by late June then disappeared by August. Our research supports these findings, as it compares to Maryland's climate zone, as well as Orstan's observations of a partial population turnover, where over-wintered young adults

emerge in spring (able to reproduce) and others are hatched in spring (non-reproducing), together comprising of the next year's breeding adults, while the large-shelled 1.5-year-old snails die off. This population cycle, coupled with *P. tenuis* L1 capability of overwintering within snails and in WTD feces, plus high precipitation events often occurring in spring (allowing optimum conditions for L1 uptake by snails), potentially equates to high larval intensity on the landscape. Additionally, Lankester and Anderson (1967) showed that snails already containing larvae can become reinfected; this would increase larval load within individual snails. In a period of three-to- four weeks (approximately starting in late June-July), L1 turn to infective L3.

3.4.3 Animal Mortality

In the second year of this study, an observation of incidence of meningeal worm related illness and death was suggested, though no necropsy was performed to confirm this. Several snails containing high and moderate larval loads were recorded shortly following grazing by a flock of sheep. Larval findings and concern for risk of illness were immediately communicated to the farmer. One ewe was reported having shown neurologic signs and died one week after exposure at this high-risk site. With their veterinarian's guidance, the farmer treated the rest of the flock with an anthelmintic as a precautionary measure. Alternative diseases with clinical neurologic signs include rabies, caprine arthritis encephalitis, polioencephalomalacia, and listeria.

3.4.4 Farm and pasture management

Pasture locations were associated with the general Succinea population, with center and verge having about double the counts of the outside region ($p<0.001$ and $p<0.01$, respectively). No significance was found between pasture sites and infected snail numbers or with larval load. *P. tenuis*-infected snails were found on all pasture site types, with fence line

locations having higher infected populations. Across all the fields tested, these areas were often bordered by mixed forest habitats. This might suggest shade as a factor of supporting microclimatic conditions for amber snails.

In observation of the six study farms, two farm locations with the highest abundance of gastropods and of infected snails had similar pasture management techniques, which differed from the other farms (as described in Chapter 5). High soil nutrient quality and microbial biodiversity were encouraged by these farmers, along with intensive rotational grazing management of sheep and goats. On these farms, animals were exposed to each section of pasture for 1-2 days and were moved as soon as the vegetation "folded over," trampled on by animals and typically left at lengths greater than 12", as opposed to grazing strategies used by other farmers when animals moved to a different section of pasture once grass heights reach 6"-8". Other farms in this study often overstocked or overgrazed their pastures, resulting in low soil moisture and low invertebrate diversity. The two experimental pastures on each of the two farms containing high-gastropod populations had similar pasture structure qualities as one another; each had one pasture with primarily grass monoculture, and each also had a pasture with diverse vegetation species, namely grass (*Phleum pratense* and *Dactylis glomerata*), dandelion (*Taraxacum officinale*), clover (*Trifolium repens and Trifolium pratense*), and burdock (*Arctium minus*). The presence of *P. tenuis* on these farms were primarily found on diverse vegetation pastures. Broad-leafed taproot plants (i.e., burdock, dandelion, nettle) may support snail populations throughout the drought season. Researchers from this study observed that the presence of morning dew allowed for the movement of snails to the tops of vegetation, which may pose increased risk to livestock grazing overnight and into the morning. Shaded areas kept dew longer on plants and often harbored higher abundances of snails. Furthermore, the climbing

behavior of Succinea snails onto tall vegetation creates opportunity for accidental ingestion by livestock (McCoy and Nudds, 1997). Observations during this study found that this type of snail does not thrive in short vegetation areas. These "hot spot" areas of shady, high moisture sites with broad-leafed taproot plants can sustain snail populations even through drought conditions, as seen by this study. This suggests *P. tenuis* can be found in pocket areas, not uniformly, across a pasture, though locations, such as the fence line, didn't make a difference in worm prevalence.

3.4.5 Limitations

This study was limited to Maine farms, though they were dispersed across the state (i.e., mid coast, inland, and mountain regions) and the diverse climates may have contributed to the range of different gastropod species documented. Additionally, deer density was not able to be calculated for each area. Relationships between deer density estimates and frequency of *P. tenuis* in terrestrial gastropods could have been compared between farms to give further insight into transmission risk.

3.4.6 Future Directions

By knowing that 97% of gastropods, and the only kind to harbor *P. tenuis*, were from the family Succinieidae, further examination of the behavior, phenology, and immunology of these snails may inform further risk assessment studies on pastures. Furthermore, comparing night and morning differences, when gastropods are reported to be most active, with mid-day presence in infected snails might inform risk reduction management strategies, such as not letting livestock graze at night. Lastly, a regional or national effort to document pasture-dwelling gastropods which harbor *P. tenuis* may be helpful for producers in areas other than Maine.

3.4.7 Conclusion

Several risk factors of *P. tenuis* were explored in this study by examining the intermediate hosts on small ruminant pastures. The recipe for risk includes large and medium sized snails of the family Succinidea that are present on grazing space in high abundance (~ 200 snails/m ^{2}). Another factor is the time of year (around two months after snail emergence), which can also influence other health problems such as heat stress in animals, thus potentially lowering immune response within small ruminant hosts. Additionally, high moisture soils with broad leafed, tap-rooted plants harbor high numbers of snails and can contain populations during unfavorable (i.e. drought) conditions. Farmers and managers may want to consider grazing animals during the day, when climbing gastropods are not as likely to be at the top of vegetation, on monoculture grassy fields, and to avoid areas with excessive numbers of amber snails.

CHAPTER 4. INTEGRATED PEST MANAGEMENT FOR THE CONTROL OF GASTROPOD VECTORS ON PASTURE

4.1 INTRODUCTION

Gastropod-borne parasites cause animal and human health challenges worldwide (Giannelli et al., 2016). Unlike free-living parasites that commonly infect livestock, *Parelaphostrongylus tenuis* (meningeal worm) and *Fasciola spp.,* require gastropod intermediate hosts to develop into their infective stage. *P tenuis*, a helminth which replicates within white-tailed deer (*Odocoileus virginianus*), is a devastating parasite to small ruminant livestock such as sheep, goats, and camelids, often causing neurological symptoms and sometimes death to these incidental hosts. Liver flukes of domestic ungulates, *Fasciola hepatica* and *F. gigantica* cause fascioliasis in people, and can stunt growth, reduce production, and cause death in livestock (Nyirenda et al, 2019). Agricultural grazing habitats are common areas of overlap between definitive, intermediate, and aberrant host, thus increasing risk of parasite infection to livestock (Wells et al., 2018). While *F. hepatica* and *F. gigantica* generally utilize aquatic molluscs as intermediate hosts, *P. tenuis* larvae are primarily found in land snails and slugs. Livestock on pastures containing abundant gastropod populations and with high use by white-tailed deer use face elevated risk of *P. tenuis* infection. Additionally, since there is no diagnostic testing for *P. tenuis* in livestock, prevention is essential to minimize risk. Disrupting the lifecycle of these parasites by targeting the gastropod intermediate host may be a solution for farmers to consider as a means of reducing disease risk to livestock.

Few studies have investigated prevention against terrestrial gastropod vectors and the parasites they carry in agricultural grazing systems. Takeuchi-Storm et al. (2017) found that

limiting animals to dry grazing areas away from wet habitat where snails might reside, reduces the risk of *F. hepatica* infection in cattle. A study which looked at the treatment effect of co-grazing goats with domestic waterfowl to prevent gastropod intermediate hosts found no significant differences in gastropod abundance after treatment, however goats alone increased abundance of gastropod hosts, suggesting the presence of ducks may keep populations from growing (Marchetto et al., 2022). In llama (*Llaama* glama) and alpaca (*Vicugna pacos*) herds, or when camelids are used as guardian animals for small ruminants, preventive measures against *P. tenuis* include routine dosing of camelids with ivermectin, though this increases the risk of anthelmintic resistance in other helminth species (Smith, 1998). Currently, there is no clear mechanism for preventing gastropod-borne parasites in agricultural settings, and more research is needed to better manage risk factors to livestock.

Integrated Pest Management

Farmers facing animal health issues due to pests and parasites may consider an integrated pest management (IPM) approach. IPM incorporates multiple control strategies through biological control, habitat modification, cultural practices, and the use of chemicals (Figure 15). Various synthetic and chemical molluscicides are marketed for snail and slug control such as bayluscide, thymol, eugenol, anilofos, fenitrothion, and copper hydroxide, though these products might not be ideal on grazing areas or organic farms (Adekiya et al, 2019; Thompson et al, 2005).

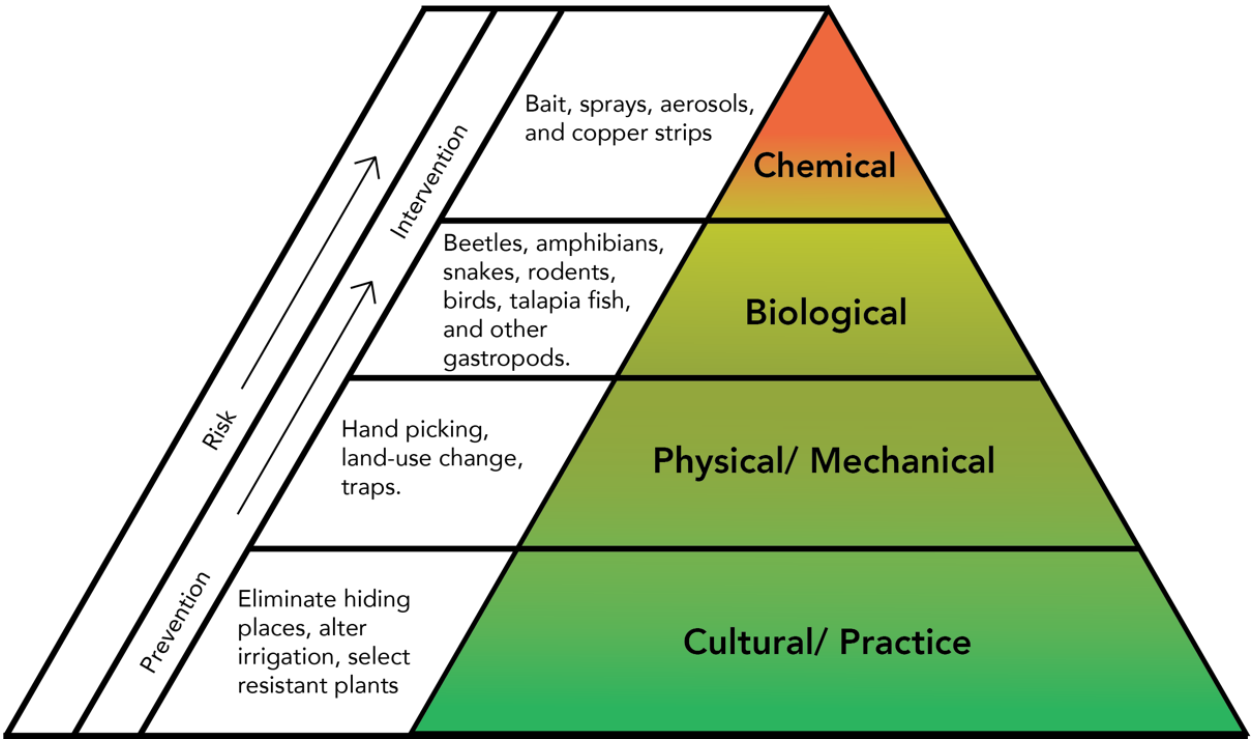

Figure 15. Integrated Pest Management (IPM) pyramid of gastropod control and prevention methods. With higher risk, stronger intervention may be required (i.e. chemicals). Adapted from the U.S. Environmental Protection Agency (EPA, 2024).

Biological control is an alternative method within the IPM framework, and perhaps more accepted within organic farming communities than chemical control means. Pastured poultry is a mitigation method against gastropods and other crop pests due to ending the life cycle of the parasite, though recent research in this area is uncommon. Clark and Gage (1999) found that chicken and geese introduced to orchards reduced the pest burden significantly. Teo (2001) compared several duck breeds' effectiveness of reducing golden apple snail (*Pomacea canaliculate*) populations in irrigated rice fields and found that some varieties, such as Khaki Campbell, known for its active foraging behavior, were more effective than docile meat types like Muscovy. Samson and Wilson (1973) found reduced *F. hepatica* snail host populations after

duck exposure to soggy pasture. Many anecdotal reports suggest guinea hens for gastropod control on terrestrial settings (Still Brooks, 2016).

IPM also offers mechanical or physical mitigation methods which often change the structure of pest habitat. Habitat modification can include eliminating shelter or breeding areas (e.g., installing water drainage, picking up debris), habitat disturbance (e.g., mowing, grazing, raking, rototilling), and installing deterrents or barriers (e.g., toxic plants, concrete barriers, salt; Whener et al.,2021; Berg, 1973). Grazing and mowing to low vegetation height can reduce soil moisture and change the structure of vegetation communities. Ausden et al. (2005) found cattle grazing on wetland reduced densities of gastropod species, which was attributed to reduced surface area of plant matter and the associated decreased humidity among vegetation. Similarly, Boschi and Baur (2007) found that horse, cattle, and sheep intensive grazing (over grazing) reduce terrestrial snail abundance and species richness, regardless of what livestock species was exposed to pasture. Mowing also kills invertebrates and repetitive mowing reduces food supply, shelter, and wintering habitat (Humbert et al., 2010). Pech et al. (2015) found that mowing twice per year in small (2m x 2m) mosaic plots can reduce snail and plant diversity by changing microclimate conditions.

The prominent gastropod family of note in this study are snails of the family Succineidae (Gastropoda: Succineidae), commonly called ambersnails, which are viable intermediate hosts for *P. tenuis* (Lankester and Anderson, 1968) and *F. hepatica* (Relf et al., 2009). Succineidae, like most land snails, favor moist environments with calcium carbonate rich soils (for shell development) as well as high humidity and high pH (Ložek 1956; Martin, 2000). Several species within Succineidae can self-fertilize, a useful life-history trait to help with survival if populations become stressed (Patterson, 1970).

Targeting parasite intermediate hosts is an emerging area of research, however few studies inspect gastropod control on a large scale, such as in pastures (Morgan et al., 2019). This case study aims to assess the treatment effects of pastured poultry and mowing on terrestrial gastropod abundance on large-scale grazing pasture systems. We hypothesize that pastured poultry and repetitive mowing will decrease terrestrial gastropod abundance on pasture.

4.2 METHODS

4.2.1 Study Area

Pastured poultry and mowing were used as treatments for this study. Two Maine farms were selected as case studies for the two treatment experiments, with poultry at one farm and mowing at another. The pastured poultry treatment site was located at a coastal farm with *Succinidae* snail populations which persist throughout the grazing season. The 4-hectare field was historically a cow pasture, surrounded by other fields, forest, and ocean. The field consisted primarily of Timothy grass (*Phleum pratense*) and red clover (*Trifolium pratense*). The mowing site, a 1-hectare field surrounded by wetland, forest, and hay fields, was at the University of Maine's J. Franklin Witter Teaching & Research Center. The location was chosen for having observed high populations of *Succinidae* snails for several years and easy access to mowing machinery. Vegetation was composed of Timothy grass (*Phleum pratense*), red clover (*Trifolium pratense*), and dandelion (*Taraxacum spp*.).

4.2.2 Pastured poultry treatment

A flock of 150 Rhode Island Red laying hens were rotated every 3-4 days in a 2,322m² electric net pasture (Figure 16). Birds had free access to layer pellet and water. Three, 10m line transects marked with flagging, within the sampling plots, were spaced 5m apart and searched

for gastropods for ten-minutes before and after birds were exposed to pasture; no gastropods were removed by researchers. Transect locations were selected randomly at least 10m from the edge of the fence and the moveable coop. Surveys were conducted in the summer of 2021. Gastropod count data were collected before (July 6, July 20, Aug 3, Aug 17, and Aug 31) and after (July 10, July 24, Aug 7, Aug 21, and Sep 4) birds were exposed to pasture with each sampling event taking place on different locations on the pasture.

Ethics statement: Due to the noninvasive nature of this data collection, including no direct contact or handling of birds by the researchers, our organization's IACUC deemed this study exempt.

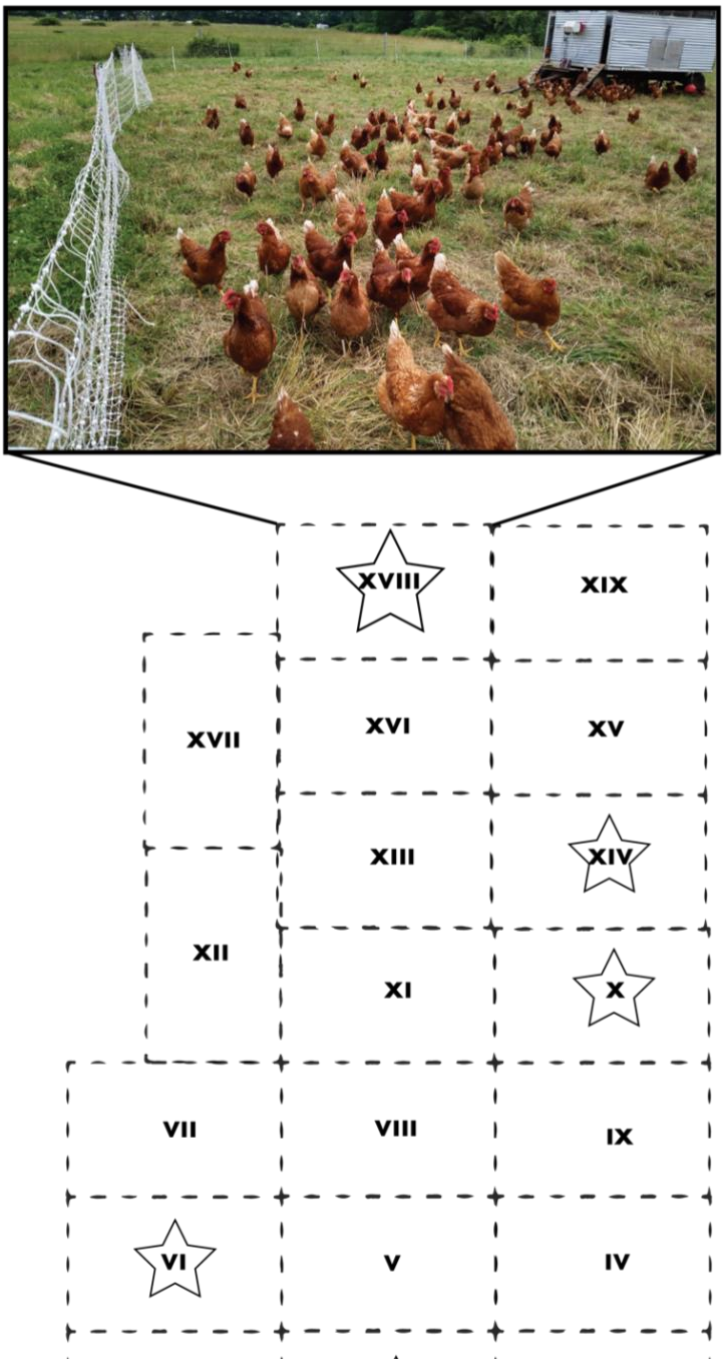

Figure 16. Chicken rotation schematic. Rotations are indicated by Roman numerals while sampling plots are represented by stars. Inset includes photo of treatment site. Sampling dates for starred locations include: II) July 6 & July 10, VI) July 20 & July 24, X) August 3 & August 7, XIV) August 17 & August 21), and XVII) August 31 & September 4.

4.2.3 Mowing treatment

Gastropod population surveys in a mow treatment field were conducted in the spring and summer of both 2021 (May 12-initial visit, June 24, July 10, August 20) and 2022 (May 20, June 6, June 20, July 5, July 17, August 4). A 4x3, 60m x 120m random complete block design (RCBD) with four blocks consisting of three, 30m x 20m plots (1-year mow, 2-year mow, and control/ not mowed) was measured and flagged (Figure 17). Mowing occurred 2 days prior to gastropod surveys and when growth reached 10-15cm (2021: May 10, May 30, June 22, July 8, August 18; 2022: May 18, June 4, June 20, July 2, July 15, August 2). A 6m perimeter was mowed to form a defined barrier throughout the study period. 1-year mow designated plots were cut in 2021 only and were allowed to grow back in 2022 to study repopulation rates of gastropods to these areas. 2-year mow designated plots were mowed for two consecutive years. Control sites were left unmowed for the duration of the study. Cut heights ranged from 3-5cm and mulch grass was not removed.

To survey for gastropod abundance within each treatment plot, we used 45cm x 90cm (Lankester & Anderson, 1972) PVC quadrats six times, spaced 5m apart at the center, to minimize edge effect. A flag was thrown to indicate a random center point for the quadrat. With each visit, sites were moved by 1m to prevent pseudo-replication. Gastropods were counted and identified to the genus level in the field; no gastropods were removed during counts. Survey elapsed time was approximately 60 minutes per block. Soil moisture was recorded at each of the six survey sites, per plot, with an Expert Gardner meter.

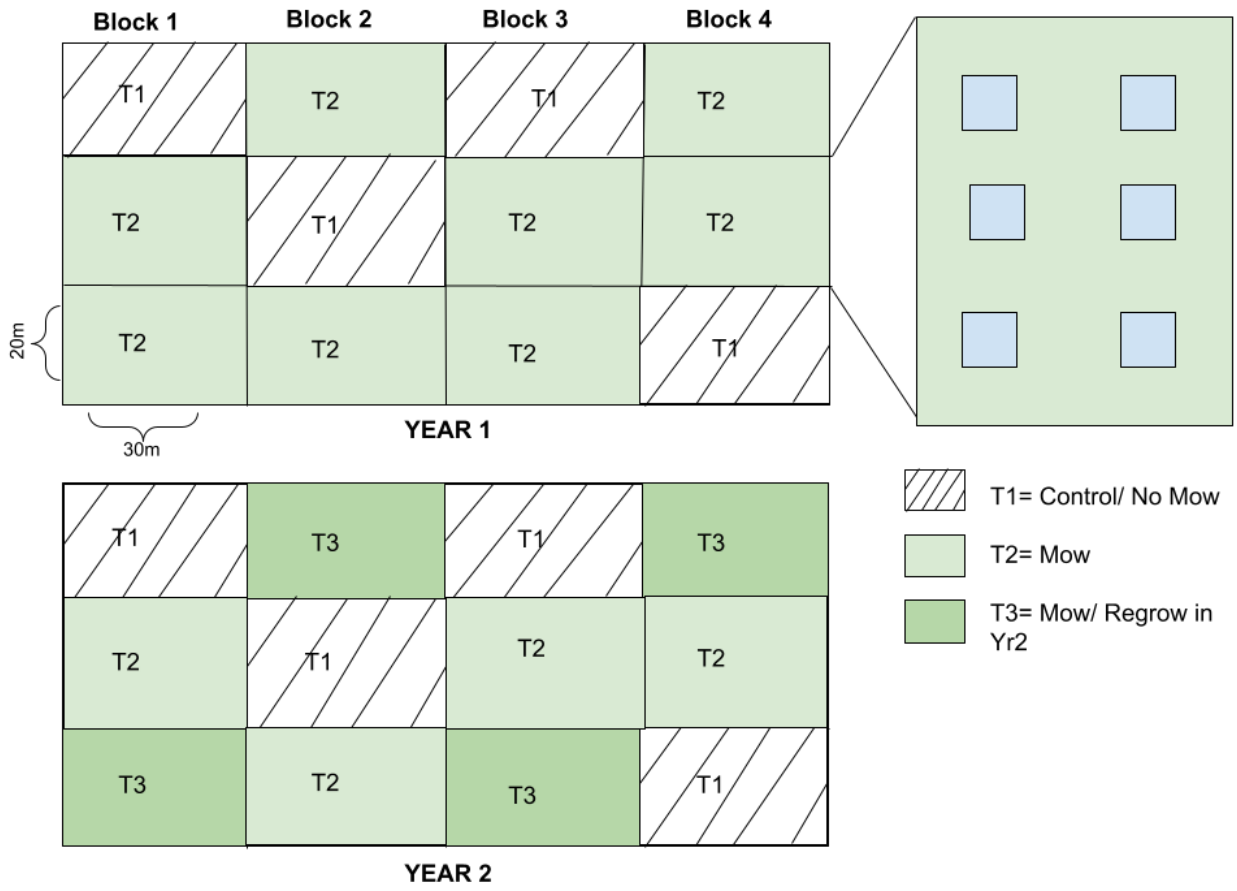

Figure 17. Random complete block design schematic of mow treatment field. T1 is control, T2 is two-year mow treatment, and T3 is one-year mow/ regrowth treatment. Plot enlargement shows quadrat placement (n=6) for gastropod sampling.

4.2.4 Data analysis

Data were analyzed using R v.4.3.0 (R Core Team 2023). All data were checked for normality, skewness, and kurtosis prior to modeling by QQ plots and histograms. Outliers were individually assessed for errors and were kept as valid points for analysis. Data sets determined to be nonnormal were modeled using nonparametric testing. Data transformation was attempted before nonparametric model application.

A paired t-test of before and after gastropod counts was performed before and after for the pastured poultry treatment. Counts consisted of the total gastropods for the three survey transects per sampling visit. Cohen's d effect size was calculated to assess practical effect of this treatment. This calculation standardizes the means of the two groups; small values, considered at or below 0.2, indicates that the mean difference is negligible, even if the differences in the groups are statistically significant, whereas values of 0.8 or more indicate that an intervention works well. A Kruskal-Wallis nonparametric test was used for both years of mow data. Dunn pairwise testing was used to determine the Benjamini-Hochberg (BH) p-adjustment. The nonparametric linear models included total gastropods ~ mow treatment + block and total gastropods ~ month. The model differed between the two years. In year one, the 2-year mow treatment did not yet exist, resulting in two levels of mowing. In 2021, we allowed the 1-year mow plots to regrow, so there were three levels (control, 1-year mow, and 2-year mow). The model to assess the effect of soil moisture on gastropod abundance was total gastropods ~ soil moisture, and to assess blocking (or spatial) and treatment effects on soil moisture, the model soil moisture~ mow treatment + block was used. Data for gastropod abundance in 2020 and 2021 were pooled to assess general effects of mowing treatments, as well as analyzed separately to see differences within years.

4.3 RESULTS

4.3.1. Poultry Treatment

Pastured poultry decreased gastropod abundance ($t(4)= 4.07$, $p=0.015$) (Table 4).. Though there were only five comparisons over the course of one grazing season, Cohen's d effect size was 1.82, indicating a very large practical significance for this treatment. Poultry

exposure gastropod values averaged 26.6 (±10.5) gastropods before exposure and 4.6 (±5.13) gastropods after exposure (Table 4; Figure 18).

Table 4. Gastropod counts before and after poultry exposure to pasture for five trials held July-September of 2021.

Survey visit	Days on pasture	Gastropod count before (mean/ SD)	Gastropod count after (mean/ SD)
1	4	12 ±6.6	0 ±0.58
2	3	6.0 ±2.5	1.7 ±1.5
3	3	11 ±3.5	0 ±0
4	4	11 ±1.5	4.0 ±3.0
5	4	4.0 ±2.6	1.3 ±1.5

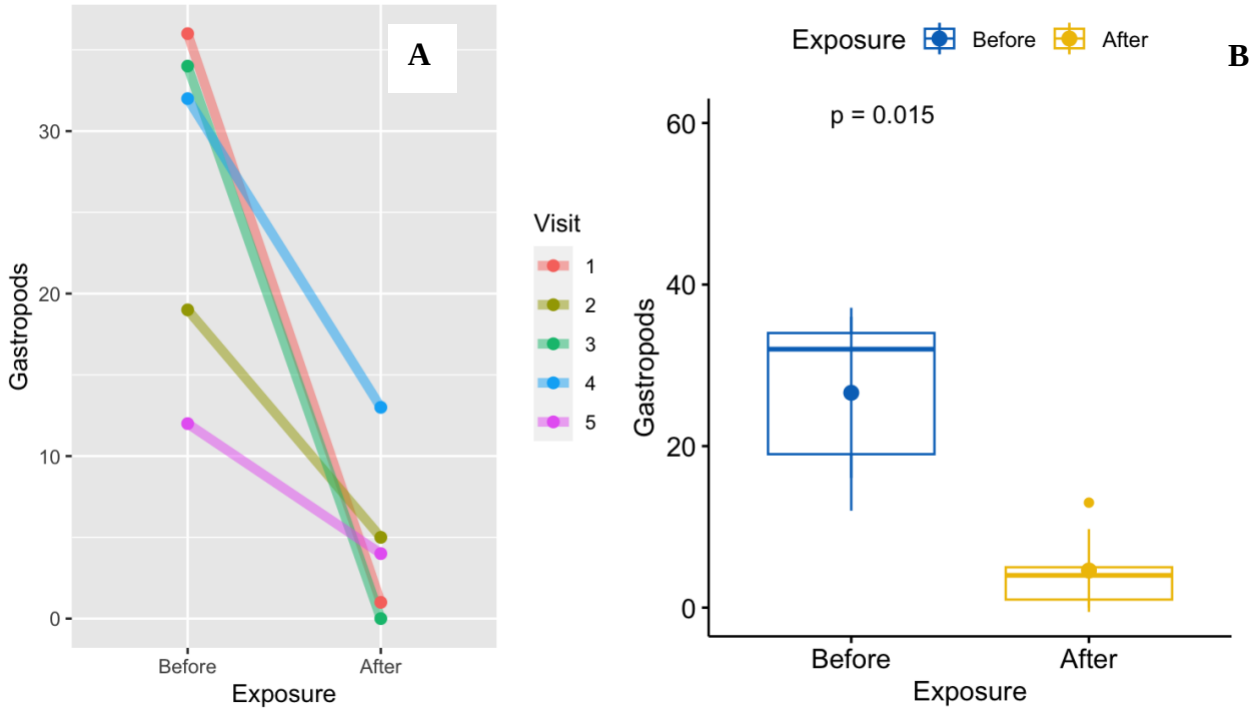

Figure 18. A) Before and after poultry exposure by sample period. B) Gastropod population box and whisker plot of before and after poultry exposure.

4.3.2 Mowing Treatment

Mowing reduced gastropod abundance compared to non-mowed sites ($p<0.05$; Figure 19). The first year, with two treatments- mow and no mow- more gastropods were found in non-mowed sites than in mowed sites ($H(2)= 23.9$, $p<0.001$). Blocking, or spatial, effects were observed in the third block which had less gastropods than the other blocks ($H(3)= 15.3$, $p<0.01$) which may be a result of accidental mowing by haying contractors.

In the second year, with three treatments, gastropod counts varied across treatments (Figure 20). The two-year mowing treatment plots had significantly less gastropods present than the no-mow treatment plots (one-year mow/regrowth + control; $H(2)= 124.4$, $p<0.001$), and the one-year mow/ regrowth had less gastropods than the control plots ($H(2)= 124.4$, $p<0.05$). The regrowth treatment plots showed a rebounding population (Figure 21.B). No significant effect from blocking on gastropod abundance was observed ($p=0.463$). Figure 20 shows monthly patterns on gastropod abundance. Visualization of year-two gastropod population seasonality of treatments and plots can be found in the Appendix (Figure A.4 and Figure A.5). Soil moisture influenced total abundance in the second year ($H(7)= 155.1$, $p<0.01$). Plots (treatments) did not have any effect on soil moisture, however blocks did ($H(3)= 13.09$, $p<0.01$) likely due to land drainage to an adjacent stream. Significance and descriptive statistic tables for mowing effects can be found in the Appendix (Table A.14, Table A.15, Table A.16, Table A.17, Table A.18), for month (Table A.19) and for soil moisture (Table A.20).

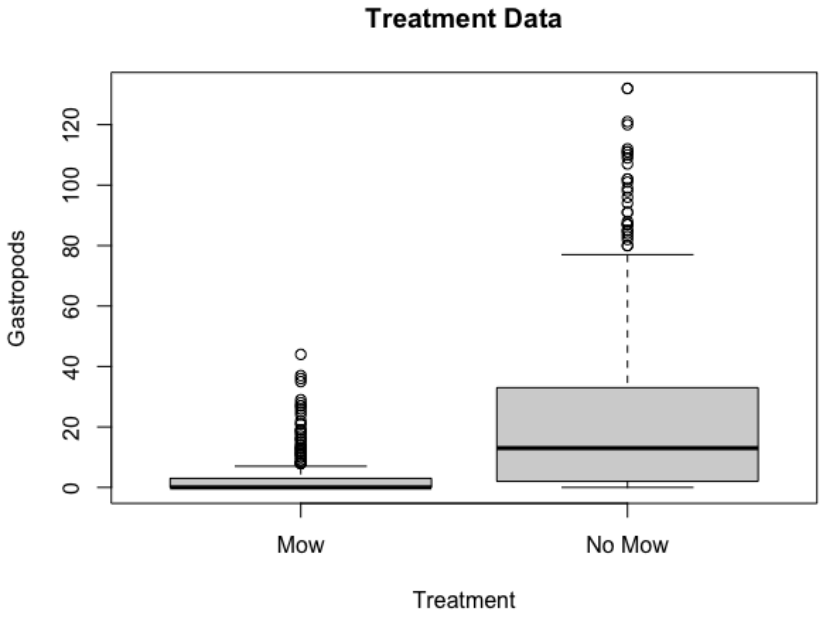

Figure 19. Mow versus no mow treatment differences in total gastropod populations for both years. Circles indicate outliers within treatments.

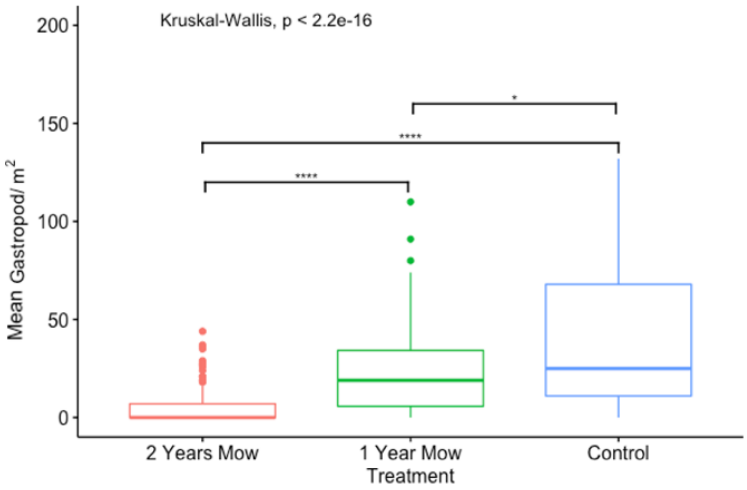

Figure 20. Year two mean gastropod counts by mow treatment. Kruskal-Wallis significance test and Dunn pairwise comparison between treatments. (*) indicates $p<0.05$; (****) indicates $p<0.001$.

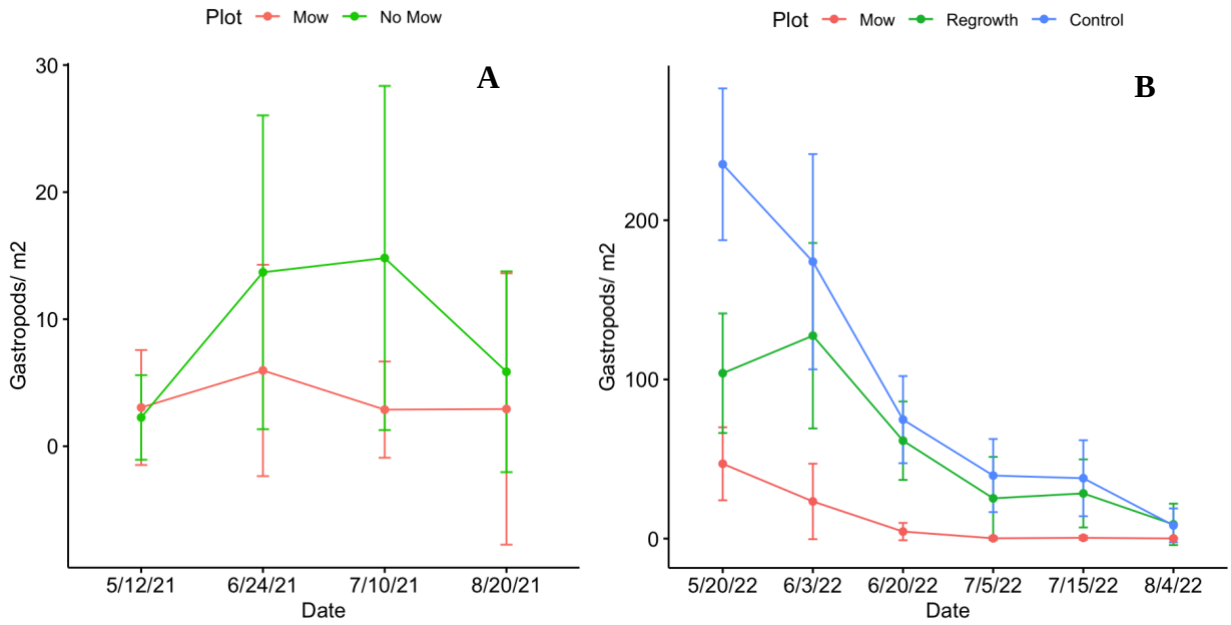

Figure 21. Monthly patterns of gastropod abundance on mow treatment plots. A) Year one gastropod population abundance by observation date with two treatments: mow and control. B) Year 2 gastropod population abundance per square meter by observation date with three treatments: one-year mow (regrowth), two-years growth, and control.

4.4 DISCUSSION

Exploring control methods for gastropod-borne parasite control in agricultural systems is important for the health of livestock animals. Targeting the intermediate hosts is one avenue for prevention for problematic parasites, such as meningeal worm. These organic pest management methods may offer strategies for farmers to consider.

4.4.1 Pastured poultry

Our research using pastured poultry for the control of gastropods shows that the rotation of laying hens across gastropod abundant areas can lower snail and slug populations, thereby

potentially reducing the risk of parasite transmission to livestock. Active poultry breeds that do well on pasture, such as Rhode Island Red chickens or Khaki Campbell ducks, may perform better than broiler chickens, although further research is needed to confirm this. The incorporation of this prevention method to pasture systems would likely require poultry exposure to pasture before ruminant grazing access. Such a process is a reversal of common IPM practices using pastured poultry, where birds are rotated after ruminants for the purpose of scratching manure into the soil and ingesting fly larvae on manure. In grazing areas with high white-tailed deer use and gastropod abundance, or a farm with a history of suspected *P. tenuis* infection in animals, farmers may want to consider rotating poultry onto pastures before introducing livestock, then, if desired, rotating them or another bird group back on once ruminates leave.

4.4.2 Mowing

Mowing as a control method for gastropods is another effective option for reducing parasite transmission. Our study showed that mowed areas contained fewer gastropods than non-mowed areas. This supports similar findings by Boschi & Baur (2007) where intensely mowed grasslands had lower snail abundance than low-intensity pastures. Vegetation that is cut or grazed short can dramatically reduce soil moisture, leading to unfavorable conditions for gastropods. Repeated mowing efforts in areas intended for grazing may not be ideal for soil health; however, in areas of high *P. tenuis* probability, this method may be ideal to practice throughout a grazing season to limit gastropod population growth. In this study, we found areas that are allowed to regrow fully after one year of mowing can quickly repopulate with snails, though we observed no egg masses or juvenile gastropods in these areas, suggesting individuals may have immigrated from neighboring control communities. Pasture-wide mowing on only one

occurrence may be effective at reducing gastropod abundance if immigration is not expected to occur from surrounding habitats, though this requires further research. This study also found seasonal effects relating to climate can assist with population decline, especially in drought conditions which further reduce soil and leaf-surface moisture.

4.4.3 Tradeoffs

The cost and time of these practices might be outweighed by the financial and emotional value of livestock and might influence producer decision making. Biologically, soil quality may degrade due to a decrease in gastropod abundance, as snails and slugs are important nutrient cyclers and engineers of soil health. Snails and slugs may be useful on farms for their role as decomposers. Decaying vegetation (and animals), as well as animal droppings, are vital food sources for many gastropods. In the process of consumption, then defecation, gastropods directly cycle nutrients from plant and animal matter to the soil. This nutrient cycling promotes growth of pasture vegetation that can be grazed by animals. Additionally, gastropods contribute to biodiversity and enhance the function of terrestrial and aquatic ecosystems. They are a food source for many insects, small mammals, fish, and bird species, which accentuates their importance for food web processes (Martin 2000). Gastropod conservation is often overlooked, but their presence can be an indicator of robust ecosystems and can predict conservation priorities for vertebrates (Lydeard et al, 2004). Their benefits expand beyond ecosystem processing to agricultural, food, and quality control. Producers may consider these tradeoffs for risk-reduction of gastropod-borne parasite transmission.

4.4.4 Limitations

This study is not without experimental caveats. Due to multiple mechanical failures in 2021, only three mow surveys were conducted and portions of two plots in the third and fourth block were accidentally hayed. Additionally, up to six white-tailed deer were observed on the field at every visit for both years, which potentially caused some disturbance or reduction of snail numbers. The statistical testing for the poultry was limited by the design, with the gastropod population in one sampling field, thus having no independence. Furthermore, the statistical tests suffer from pseudoreplication as each type of treatment was evaluated in one field each. The effectiveness of both treatment types should be evaluated further by using multiple locations which include diverse soil and environmental conditions to assess the applicability of the two treatment options to farms more broadly.

4.4.5 Future directions

Methods to further explore effectiveness of the treatments in this study could expand our findings by comparing different breeds of poultry, mowing at different height intervals, and combining mowing and poultry on snail-abundant sites.

This study explores the practical use of pastured hens and repetitive mowing on gastropod-abundant pasture where risk of gastropod-borne parasite transmission is high. These treatments are organic, reducing the possible toxic repercussions of chemical molluscicides to livestock on grazing space. Future experimentation of long-term gastropod population reduction impacts, both on animal and soil health, are worth investigating.

CHAPTER 5. SMALL RUMINANT HEALTH, PARASITE RISK, AND INFORMATION EXCHANGE: STAKEHOLDER KNOWLEDGE, ATTITUDES AND PRACTICES

5.1 INTRODUCTION

Animal caretakers, such as farmers and veterinarians, make decisions about animal health based on several methods, such as prior education and experience, consulting mentors and peers, and more recently, the internet and social media (Ellis-Iversen et al., 2010, Roybal, 2012; Alarcon et al., 2014; Pires et al., 2019; Svensson et al, 2019) These routes of information may influence how farmers perceive animal health and parasitic risk and thus may cause challenges for responding veterinarians (Kogan et al., 2014; Kogan et al., 2017; Shortal et al., 2018). Small ruminant veterinarians (those providing services for llamas, alpacas, sheep and goats) have multifaceted jobs; not only do they care for their animal patients, but they also serve as educators for farmers at all education levels and production scales. Examining information feedback loops about parasites, zoonotic diseases, and general health management may highlight knowledge gaps in educational programming.

Farmers who have good relationships with their veterinarians often reach out to them as a primary source to seek advice and information on animal health, thus creating high levels of trust between vets and farmers (Gunn et al. 2008; Garforth et al. 2013; Ruston et al. 2016). These veterinarian-client-patient relationships (VCPR) between farmers and veterinarians enhance sustainable and efficient health management strategies; reasons for not establishing a VCPR include producer economic constraints and veterinarian availability (Lee et al., 2022). Replacement of a VCPR with animal health misinformation, often found online, can result in management “firestorms” (Pfeffer et al, 2014). This effect can cause challenges for veterinarians trying to remedy the poor health management practices of producers. Good relationships with

clients allow veterinarians to transition from a focus on individual animals to a whole herd health approach. Shifting from a '"test and treat" model to a "predict and prevent" model allows the veterinarians to have a more robust outlook on disease and parasite impacts on herd/flock productivity (Barkema et al. 2015; Brockett et al., 2021).

In small ruminant production, parasites are one of the top contributing factors to poor performance and mortality. Nematodes, such as *Haemonchus contortus* (barber pole worm) and *Parelaphostrongylus tenuis* (meningeal worm), can cause illness and death of small ruminants, reducing productivity and profitability. In the case of meningeal worm, since it cannot reproduce in aberrant hosts, such as domestic small ruminants, there is no premortem diagnostic test to help farmers and veterinarians with management or treatment decisions. Rather, worms migrating in the nervous system may be found at necropsy, or a presumptive diagnosis is made by ruling out other causes of neurological symptoms and/or a response to anthelmintic treatment. In general, parasitic risk to animals varies from farm to farm due to variables including management and environmental differences. Additional factors such as a changing climate and parasites' increasing anthelmintic resistance of parasites highlight the need for alternative parasite control strategies By adopting a "predict and prevent" mindset about parasite management, farmers may lower risk of infection in their livestock (Taylor, 2013).

Scientific learning opportunities can have mixed effects within the working farmer community, and advice on ways to improve practices may or may not get adopted. For social, economic, and physical reasons, farmers may not heed scientific advice (Higgins et al., 2012, Brocket et al., 2021). When given the opportunity to contribute to scientific understanding, farmers may be more willing to adopt these practices. Combining local knowledge (i.e., farmers)

with scientific knowledge (i.e., veterinarians and Extension) can improve acceptance of recommendations, enhancing long-term sustainability (Mantyka-Pringle et al, 2017).

The aim of this project was to record local knowledge about small ruminant producers' animal health management and scientific knowledge about parasite and health management strategies, challenges, and needs based on the perspectives of farmers and veterinarians. This information may help inform managers and veterinarians about sustainable solutions to animal health and parasite control, in addition to capturing emergent themes related to knowledge gathering and sharing preferences.

5.2 METHODS

5.2.1 Participant Recruitment

Stakeholders included small ruminant farmers and practicing large animal veterinarians practicing in the state of Maine. For this report, six producers, who were previously involved in a separate on-farm research experiment analyzing risk and prevalence of *P. tenuis*, were interviewed, in addition to four clinical veterinarians active in small ruminant practice.

5.2.2 Sample Collection

In the winter of 2022-2023, farmers and veterinarians were asked a series of questions via individual, semi-structured interviews; each group was asked a unique set of questions (see supplemental documents in Appendix B and Appendix C). This format, and the open-ended structure of several questions, allowed participants to expand freely on their answers and to bring up any content related to the topics of the study. Interviews were held in person or virtually and audio was recorded. The transcription software Dovetail (Dovetail Research Pty. Ltd.) was used

to transcribe conversations. Interview methods and content were approved by the University of Maine Institutional Review Board; all data are confidential.

Prior to the interviews reported in this study, farmers were presented with their farm-specific results from the previous two years *of P .tenuis* risk assessment These results included prevalence of *P. tenuis* intermediate hosts (snails and slugs) on pastures, prevalence of *P. tenuis* larva within those hosts, a heat map of pasture risk derived from prevalence data and other risk factors, comprising a rating of overall risk across the grazing area (i.e., low, moderate, moderate-high, high), and recommendations for risk mitigation. A pre-interview discussion also included a synopsis of the research results across all farms; however, farm identities and specific locations were not revealed. A similar synopsis was shared with the 4 veterinarians prior to their interviews. For both groups, we asked questions about their previous and current knowledge, attitudes, and any management changes that might stem from these data.

Actual risk was assessed in a separate study from May to September of 2021 and 2022; risk reduction methods were studied only during the summer of 2021. During those studies, methods for risk analysis included a bi-monthly visit to each respective farm to assess gastropod population measurements on livestock grazing spaces frequented by white-tailed deer (*Odocoileus virginianus*; WTD; *P. tenuis* definitive host) and to document host habitat and climatic variables. The risk reduction study methods included pasturing laying hens on or mowing of high-risk pastures; both were effective methods of gastropod host reduction (see Chapter 4).

5.2.3 Analysis

Qualitative analysis included combining targeted (structured) and emergent (unstructured) themes from stakeholder conversations. Topics were analyzed in the context of

real-world experiences, with an inductive thematic approach to the data (Patton, 1990; Braun, 2006). Dovetail transcription software enabled categorization of topics and key words highlighted in recorded conversations. Sentences or whole paragraphs that corresponded to concepts or beliefs within the realm of our question outline were coded by the first author. After all transcripts were coded, codes were merged based on question number or concept. Transcripts were reread to confirm that the context of content accurately represented the data.

5.3 RESULTS

5.3.1 Characteristics of Participants

Participants profiles are found in Table 5 and Table 6. Reported purposes for keeping animals included meat and/or wool production, dairy, breeding stock, education, and agrotourism. Veterinarian interviewees included three women and one man. Farmer interviewees included three women and three men.

Table 5. Farmer profiles. VCPR is veterinary-client-patient-relationship.

Farmer ID	# Years of Sheep/ Goat Care Experience	Herd Size (max)	Has VCPR
A	42	200	**Y**
B	2	30	**Y**
C	7	40	**Y**
D	25	200	**N**
E	55	120	**Y**
F	29	180	**Y**

Table 6. Veterinarian profiles.

Vet ID	# Years of Practice	Owns Livestock
A	11	Y
B	15	Y
C	4	Y
D	2	N

5.3.2 General Animal Health Management Practices by Farmers

Responses indicated that all farmers interviewed cared deeply about the health of their animals and adopted multiple practices of to maintain good herd/flock health (Figure 22). All reported observing animals daily. Other reported farmer practices used included offering free-choice minerals (100%), frequent body condition scoring (83%), annual vaccinations (83%), and periodic selenium supplementation or annual selenium injection (33%).

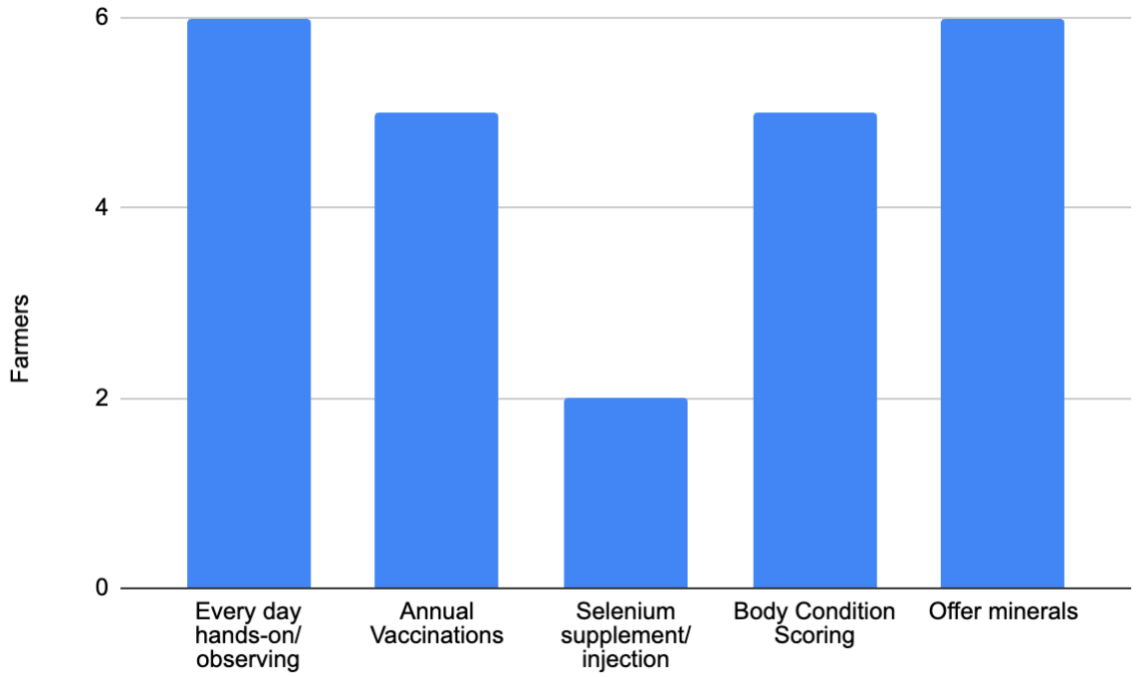

Figure 22. Farmer reported animal health practices.

All farmers claim to value advice from veterinarians, though the group had differing levels of veterinary involvement (Table 5, VCPR). Two farmers with the most animals only utilize a vet for extreme emergencies (e.g., cesarian sections, hard prolapse situations) and rely on human medical professionals for advice on treatments before seeking veterinary care. Two farmers with 120 to 150 animals occasionally have a vet come out, but only for biosecurity

panels or emergencies and claim that they have strong VCPRs which allow them to acquire over-the-phone advice from their veterinarians before mutually deciding whether the event is worth a visit to the farm. These four farmers incorporated a "survival of the fittest" model; animals requiring health care were culled., Each producer cited a unique threshold for culling (e.g., emotional connection to an individual animal, economic limits to veterinary expenditures, repeated illness in one animal). Two farmers with smaller herds/flocks use veterinarians multiple times a year for blood draws and pregnancy checks, in addition to annual check-ups and vaccinations. These farmers had fewer years of experience in years and would be considered new farmers by the USDA definition (USDA, 2024).

5.3.3 Themes

Our interviews with stakeholders included prompts about knowledge, perceptions, and adoption of animal health best practices. The use of open-ended questions allowed participants to freely expand on their experiences. Analysis generated themes of parasite and zoonotic disease risk, animal health information exchange, and challenges of best practice implementation.

Theme 1: Knowledge, perception, and management of small ruminant parasites and zoonotic disease risk

Subtheme 1.1 Meningeal worm risk to small ruminants

Prior to this study, four of six farmers had heard of or experienced meningeal worm on their farm. After learning the results of this study, most of the farmers reported a change in their knowledge, attitudes and practices regarding *P. tenuis*. At the beginning of the separate risk study (see Chapter 3), farmers were described as having low (66%), moderate (17%), or high (17%) perceived risk of meningeal worm transmission to their livestock. After seeing their farm-

specific result and a heat map of risk on their pastures, all farmers reported a change in knowledge about *P. tenuis*, notably about life-cycle and intermediate host ecology.

Results of a study of gastropod treatments on pastures using poultry and mowing techniques were shared with participants. All veterinarians said they would consider sharing these methods with farmers experiencing meningeal worm-related illness in their livestock. All farmers implemented management policies consistent with the level of actual risk detected by on-site evaluation for *P. tenuis* intermediate hosts on their farm (Table 7). *= *No prior knowledge of the parasite.*

Table 7. Producer-perceived vs. actual risk of *P. tenuis* and reported KAP post-sharing on-farm risk analysis results. *= No prior knowledge of the parasite.

Producer	**Perceived risk**	**Actual risk**	**Knowledge change**	**Attitude change**	**Change in management**
A	Moderate-High	Low	Yes	Yes	Yes; target of intermediate host plant habitat via mowing, install fence to limit WTD movement
B	Low*	High	Yes	Yes	Yes; target of intermediate host plant habitat via spraying and mowing, addition of pastured poultry
C	Low	Moderate	Yes	Yes	Yes; Addition of pastured poultry
D	Low	Low	Yes	No	No
E	Low*	High	Yes	Yes	Yes; target of intermediate host plant habitat via mowing, addition of pastured poultry, install fence to limit WTD movement
F	Moderate	High	Yes	Yes	Yes; target of intermediate host plant habitat via mowing

All veterinarians reported meningeal worm cases in practice, but cited challenges ruling out differential diagnoses, some of which are serious/potentially zoonotic (transmissible between animals and humans), citing a lack of producer support (expenses and time) for diagnostic testing to rule out differential diagnoses (Table 8, Theme 1.1.A).

Table 8. Theme 1.1: Responses from stakeholder respondents regarding *P. tenuis* risk and challenges.

Theme 1.1	Participant Response
A. *P. tenuis* diagnostic challenges for veterinarians	• Vet A: "Yeah, we definitely have suspected cases. I've never been able to diagnostically confirm a case." • Vet C: "I tend to down go down the differential list and of course rabies is always on with neurological signs. Have I ever actually diagnosed the worm? No, I haven't. I will say some of the animals got better, some of them died. So it's like, 'maybe one of these things will work'- throw a bunch of stuff at the wall and see what sticks…It's a precarious situation that kind of leads to the shotgun approach to treatment and skipping of diagnostics with the very time critical, time sensitive nature of neurological processes in these animals and then [the farmer's] money concerns related to that testing." • Vet D: "I almost never diagnose it. It is almost always a diagnosis by resolution based on treatment because people don't want to spend the money on diagnosis. It's much, much cheaper just to give the slew of neurological medicine and kind of hope it works out from there. But usually when I see a neurological goat, there's like four things that you think about right off the top of your head. One of them is rabies, another one is CAE, a third one's polio, the fourth one's listeria. And then the fifth one is *P. tenuis*. And the three that we can treat, we usually all treat at the same time because it's like $20 to do that rather than to go through and diagnose everything. As a clinician, that frustrates me, I'll be honest, but there's just not a push for diagnosis from producers."
B. *P. tenuis* not a part of routine animal health conversations	• Vet C: "It's a topic that's not very commonly broached, unfortunately, because of all of the other basic parasite, nutrition, and vaccination information I need to give. It gets lost and it's not prioritized." • Vet D: "I will say I do not routinely talk about that. I have to be selective and talk about the higher priority areas."

When asked about their conversations with farmers assessing risk of, and taking preventative methods against *P. tenuis*, three of the four veterinarians reported not routinely discussing meningeal worm with clients, except with camelid owners (Table 8, Theme 1.1.B). Llamas and alpacas are thought to be highly sensitive to meningeal worm (Ismail et al., 2011). With clients who own other small ruminant species, concerns about other internal parasites, primarily *H. contortus*, take precedence as it is the most common parasite they treat and advise farmers on. Generally, only when suspected cases were detected would they discuss *P. tenuis* with their clients.

Subtheme 1.2 Small ruminant parasites

Both farmers and veterinarians were asked about the frequency with which they observe parasitic illness within small ruminants (Table 9). All veterinarians reported that parasite-related illnesses are extremely common and observed more often in small ruminants than in cattle or equids. All the farmers reported to have had past or current problems with parasites, namely *H. contortus*, coccidia, lice, and *P. tenuis*.

All participants were asked to rate their concern about anthelmintic (chemical deworming) resistance in parasites (1= not at all concerned, 2= somewhat concerned, 3= very concerned). All of the veterinarians were very concerned; 60% of farmers were very concerned while 40% were somewhat concerned.

Table 9. Theme 1.2: Stakeholder responses about parasitic anthelmintic resistance concern.

Theme 1.2	Participant Response
Concern about parasite anthelmintic resistance	• Farmer A: "I am very concerned because there aren't a lot of deworming options out there. So I don't want to lose faith in my options." • Farmer E: "One has to keep changing one's drench on a regularly basis and being very careful not to over-drench. So there are all sorts of ways in which this can become a major problem of parasites and it's a bad way of controlling them in general. Plus, the internal organs, which I often like to eat, concentrate these worms- as I say, a whole bunch of reasons for human health and animal health why I think resistance is a really big concern." • Vet A: "In order to effectively treat any of these things that we're talking about, you have to use off-label dewormers, which means that you're really supposed to be doing that with veterinary oversight because off-label drug use is legally supposed to be done with a veterinary prescription." • Vet B: "We have resistance already regardless of what species we're dealing with. We know that most sheep and goats are resistant to most anthelmintics out there. To the point where now we have to give them a borderline toxic dose to make any of these medications effective. We have to start thinking outside the box and again, fasting them, FAMACHA scoring with a retest of the burden of the eggs… We're essentially using medications that could be toxic to fetuses in order to treat these animals for parasites and not even be able to effectively kill all the parasites that we're dealing with."

Farmers were asked to discuss their general parasite management practices which were then compared with the recommendations given by the veterinarians in this study (Table 10). Veterinarians reported advising farmers to use a range of management methods, ideally using multiple methods. These included fecal egg count (FEC), FAMACHA (i.e., visual scoring of anemic index) scoring followed by selective treatment of only the affected animals, using more than one class of anthelmintic when treating, rotational grazing (and avoiding grazing on very short vegetation), frequently surveilling for symptoms suggesting parasite burden (e.g., slow growth, weight loss, etc.), and using culling and selective breeding practices to build genetic

resistance to/ tolerance of endemic parasites. All veterinarians advised that these methods be prioritized over routinely scheduled deworming to prevent anthelmintic resistance by parasites. However, three out of four veterinarians also suggested anthelmintics be given to camelids monthly (i.e., llamas and alpacas; often co-pastured with sheep and goats) due to their heightened sensitivity to meningeal worm-induced damage. Parasite management practices varied among farmers; most adopted multiple practices that aligned with veterinarian recommendations (Table 10).

Table 10. Participant-reported parasite management recommendations and practices. Total participants: Veterinarian n=4; Farmer n=6.

Parasite management method	Farmers Practicing (n=)	Veterinarians Recommending (n=)
Diagnostics		
• Fecal Egg Counts (FEC)	1	3
• FAMACHA then treat	6	4
• Symptomatic then treat (e.g., ill thrift, diarrhea)	6	3
Treatment		
• Scheduled deworming (excluding camelids)	0	0
• Dose with 2 classes of anthelmintic treatment	1	4
Control/ Prevention		
• Cull & build genetic resistance/ tolerance	4	4
• Rotational grazing	6	3
• Natural anthelmintics as preventatives	1	0
• Co-grazing other livestock on shared pasture	2	2

Subtheme 1.3 Zoonotic disease risk

Participants were asked to rate their concern (same scale as anthelmintic resistance, above) about zoonotic pathogen or parasite risk. Farmers ranged from not at all (20%), slightly (60%), to very (20%) concerned about zoonotic pathogens. When asked for the reasoning supporting their rankings, those who were not or only slightly concerned stated they had no knowledge of or had not been exposed to zoonotic pathogens/parasites (Table 11, Theme 1.3.A).

Half of veterinarians were very concerned, 25% were somewhat concerned and 25% were not at all concerned about zoonoses. Veterinarians who were very concerned claimed it was due to their personal high risk of exposure (Table 11, Theme 1.3.B). Half of the veterinarians reported that they mention zoonotic pathogen risk during every farm visit.

Table 11. Theme 1.3; Responses from stakeholder respondents about zoonotic disease concern.

Theme 1.3	Participant Response
A. Farmer understanding about zoonotic parasites or pathogens	• Farmer A: "I haven't really experienced those things and so they're not really on my radar yet." • Farmer E: "I'm concerned, but not very knowledgeable."
B. Veterinarian concern about zoonotic parasites or pathogens	• Vet A: "I think that at this point I sort of feel comfortable with what is zoonotic and we have those discussions. And for the most part, I really haven't seen much. I feel like it's pretty avoidable, like, it's not hard to not get these diseases if you're smart and educated about it." • Vet C: "I think a competent immune system is a wonderful thing. Healthy people, competent immune systems are wonderful things. Not everybody is fortunate enough to have that, and that's why we have to worry about zoonosis." • Vet D: "I am very concerned for myself, honestly more than anyone else. I would say I'm a three for me and a two for other people… We're interacting with blood and their fluids and it happens so often that I don't even think about it. And so, you know, you have blood on your hand, then you bite a sandwich and you don't even think about it until it's too late. I have never once put a glove on for a goat or sheep dystocia just because it doesn't work out as well. I think veterinarians are more in these high-risk situations like that."

Theme 2: Information exchange about animal health topics

Subtheme 2.1 Farmer animal health information sources

Farmers were asked to list their sources of animal health information (Figure 23). The most utilized by all farmers was the internet (i.e., search engine). Veterinarian advice and reading books were the second most used sources, followed by Facebook groups, peers or mentors, peer-reviewed literature and Extension literature. Webinars, list-serves and local associations were used the least.

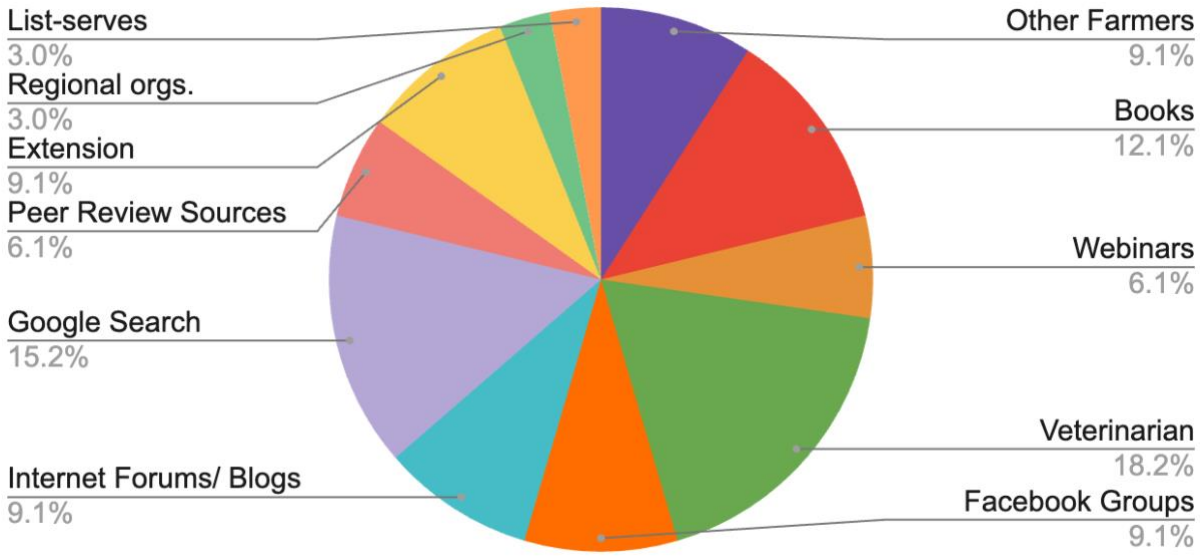

Figure 23. Pie chart of animal health information source use based on farmer response.

Farmers vary in their opinions about, and use of social media and the internet as information sources. Half of these farmers reported giving advice on social media but having little trust in it as a resource. Four out of six farmers cross-reference information when using internet searches and/or social media to inform their management decisions (Table 12, Theme 2.1.A). Farmers expressed a need for other information sources, such as peer and professional networking (Table 12, Theme 2.1.B).

Table 12. Theme 2.1: Responses from farmers about animal health information resources.

Theme 2.1	Participant Response
A. Farmer use of social media and internet for animal health information	• Farmer B: "If two people say similar things, I will give it a try." • Farmer C: "I do multiple searches, take the average of that knowledge, then make a decision based on that." • Farmer D: "You know, it used to be books or word of mouth, of course. But that's harder and harder to do. You know, there's not a lot of sheep farmers that have more than just pets to get advice for large operations. There's a lot of good information online. So I look at that, somewhat, but generally if there's a problem I call a trusted friend that has even more experience than me or do a Google search. Or call the vet and the vet goes to Google."
B. Need for local networking and information sharing	• Farmer A: "Farmers need a platform where we can share what issues we are having in relation to the same weather and other similar variables. Quarterly meetings with local farmers and scientific professionals would be so helpful." • Farmer F: "It would be great to connect with others and share these [animal health] experiences and see what they've experienced, specifically with systems-based approaches."

Subtheme 2.2 Veterinarian communication barriers

Veterinarians were asked to describe what they needed to enhance their communication about management for farmers regarding parasites and animal health. All veterinarians reported that most clients are small farm operations and/or hobby farmers, many of whom lack basic animal husbandry education. All of the veterinarians in this study wished for more educational resources that they could direct their clients (Table 13, Theme 2.2.A). All reported to frequently refer clients to scientific online sources, notably Extension websites, though 75% of veterinarians wanted to have access to physical handouts to give to new farmers. Two of the four veterinarians mentioned a desire for tutorials and credential programs on basic care and parasite management to which they could refer clients.

All veterinarians experienced communication limitations with new farmers, largely due to the overwhelming amount of information delivered during time-constrained visits (Table 13, Theme 2.2.B). All veterinarians mentioned competing with, or dealing with the repercussions from misinformation, namely advice from farmer-to-farmer or social media sources (Table 13, Theme 2.2.C).

Table 13. Theme 2.2: Responses from stakeholders regarding veterinarians' communication needs and barriers.

Theme 2.2	Participant Response
A. More educational resources for basic animal health and husbandry best practices	• Vet A: "I think it would be great to have more client education materials which you could just send to people that were easy to understand, like a one- or two-page handout. I would definitely support more sort of client education resources through the Extension service because I considered that to be a very reliable source that I can recommend to people." • Vet B: "It's kind of overwhelming as a veterinarian trying to educate someone on basic goat, sheep, alpaca management and address all they need to do to prevent the forest fire that they have created. These issues prevent us from reaching larger goals like herd growth or production. So I think as far as something that could help these kind of clients is some sort of brochure or chart to be able to give them for basic ways they can help their pasture improve and for parasite management." • Vet C: "We need interactive tidbits or tutorials that can be sort of computer-based learning where they could go in and really assess their learning and comprehension at the end of it, say with a little quiz or something like that. Or maybe gain some sort of certification once they complete these things. Have it be a consistent and accurate source of information that we all can sort of point small ruminant clients towards. Once we're all speaking the same language, then the communication becomes entirely more efficient. Then we can go out to address herd health and how to maximize productivity."
B. Limited time for animal health education at farm visits	• Vet B: "New and hobby farmers get hit with a lot of information during my visits, especially for initial herd health evaluations. They get a glazed look in their eyes, so I try to cover the basics: body condition scoring, FAMACHA, pasture rotation, and nutrition."

Table 13 continued	
	• Vet D: "When I talk about things with producers, they seem to take home probably 10% of what I'm saying."
C. Misinformation effects on animal care	• Vet A: "A problem with parasite issues is that a lot of the research and recommendations are really new. A lot of people are talking to their old farmer friends who aren't staying on top of it…We get people who call all the time that are not clients and a lot of time they've already looked on the internet and on forums. They maybe have already dewormed their animal and with something based on that research and the sources that people use, or what they find first, don't tend to be very reliable sources." • Vet C: "There is so much information out there on the web and Facebook groups that these small ruminant producers are kind of utilizing each other for that exchange of information and they try multiple things. They try to handle it on their own and sometimes some of them do very well and sometimes they don't do as well. And then I get called at two o'clock in the morning, animal down, start of death, nobody knows what's going on and probably will never know what's going on. That's usually that's a euthanasia kind of thing…It leads to a lot of confusion and a lot of lack of confidence in the veterinarian." • Vet D: "I hear a lot of things that are like, 'well my neighbor said', or 'the breeder said', or 'I saw on Facebook'… Another big one that happens is people do not understand body condition scoring of goats. The number one emergency that we see at this practice, second to none, is an emaciated starving goat. Not because people are purposely starving their animals, but because they don't understand that that goat is skinny and someone might have told them that the goat is fat because it's fluffy. There's misinformation out there. I'm assuming it comes from the internet or like a neighbor and it's super frustrating. It also extends to drugs. People get drugs from other places and I'm like, 'You're a client of ours. I've seen you every other month for like the past two years. Where did you get that?' I guess a long story short, I see it a lot and the very, very real outcomes of it."

Theme 3: Challenges to implementation of animal health best practices

Veterinarian feedback

It is evident from the previous theme that a lack of basic animal husbandry knowledge combined with misinformed practice adoption by farmers impacted the ability to provide effective veterinary services. Additionally, barriers described by the veterinarians (Figure 24) contribute to a positive feedback loop: since many farmers don't have a VCPR, they tend to make their own diagnosis and treatment decisions, and this often results in emergency veterinary intervention, which is often "too little too late" and may end in euthanasia. This poor outcome then contributes to both the veterinarian and the farmer's lack of confidence, thus starting the cycle over.

All veterinarians commented that the lack of relationships with farmers was correlated with low effectiveness of animal response to treatments and the creation of the exacerbation of animal health problems on farms (Table 14, Theme 3.A). Lack of record keeping by farmers was another challenge veterinarians mentioned (Table 14, Theme 3.B).

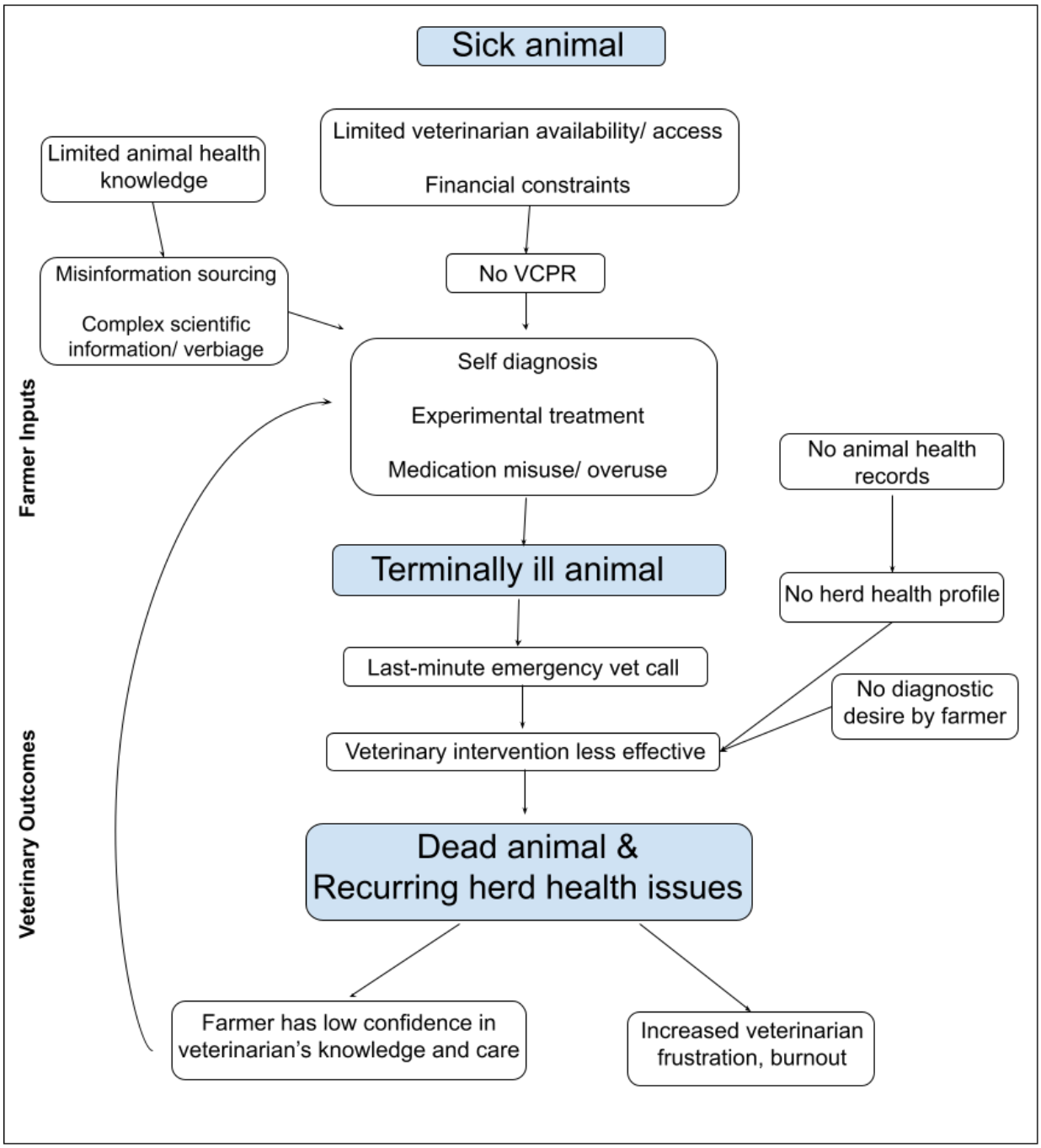

Figure 24. Positive feedback loop of animal health management firestorms (content informed by veterinary stakeholders). Farmer inputs impact veterinary outcomes, leading to a cycle of poor animal health decision-making on the part of the farmer.

Table 14. Theme 3: Responses from veterinarians regarding barriers to animal health.

Theme 3	Participant Response
A. Lack of VCPR creates extended health problems for farmers	• Vet A: "[Non-client emergency] situations get frustrating and I don't know that there's necessarily a perfect solution for that because you can't reach out to people that you don't already have a connection with. When it really starts to go downhill, that's when they call. And at that point the effectiveness of your treatment plan is not as good as if you caught it earlier. I think the people who we have relationships with call earlier because we've had a relationship with them and we've talked about this kind of stuff. So those are going to have better outcomes and I think the client education part of it has already been done, at least to the degree that they know that they should reach out." • Vet B: "Really the biggest thing is compliance and follow up. I'd probably say that is my biggest struggle with these guys. They'll usually have me come out and put out whatever fire they're dealing with, and then they'll kind of forget about it and I'll never hear from them again." • Vet C: "I have clients that call up and say 'Hey, I want to establish a vet client patient relationship with you." And then they say, "Okay, well here's a, here's a laundry list of medications that my Facebook friends say I need."
B. Lack of animal health records create barrier for whole-herd health profiling	• Vet B: "Another struggle I have is getting people to keep track of their animals. I go to farms and ask to see their records, and they just say everything is in their brain. That isn't helpful for me to get a herd health profile." • Vet D: "I would say there's an extraordinary low percentage of people who keep records and identifiers on animals. It's really hard to see which populations are affected because we don't know how old they are. We don't know how many babies they've had. We don't know if they've had any health problems in the past. We don't know if they've suddenly lost weight or not. We have no idea what their previous body condition score is. It's hard to have a big picture look at a herd from a management perspective without individual identifiers and records of things like that"

Farmer feedback

In the interviews, farmer stakeholders freely brought up management challenges and needs regarding animal health best practices, listed in Figure 25.

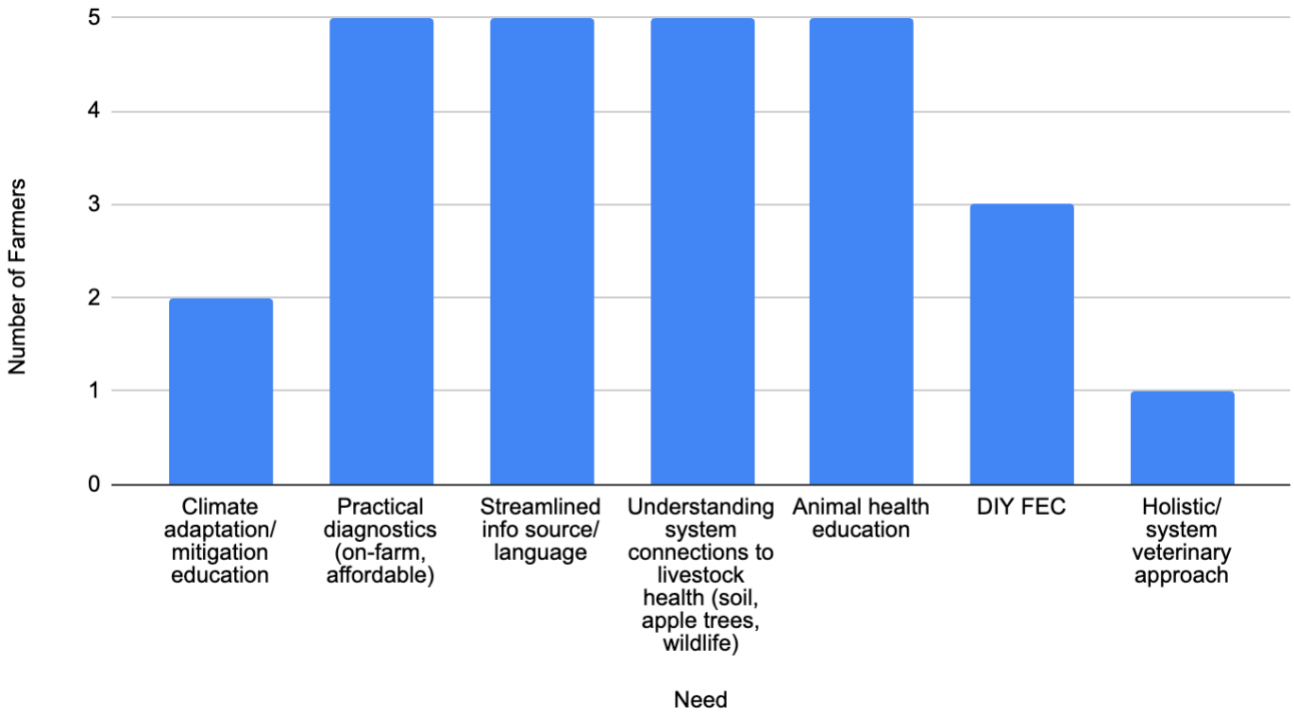

Figure 25. Farmer responses regarding needs for animal health practice improvement. Six farmers interviewed; topics derived from coded conversations about animal health management practice improvement.

5.4 DISCUSSION

This research focused on the knowledge, perceptions, and methods of farmers and clinical veterinarians regarding small ruminant health management practices. These findings highlight the complex and diverse dynamics of management decision-making by farmers, and of the relationships between veterinarians and livestock producers.

Factors such as lived experience, perceived risk of illness, and alternative information sourcing by farmers can influence these dynamics of decision making. This study shows that site-specific data collection and result sharing can improve farmer and veterinarian knowledge, attitude, and management practices concerning parasites. Farmers greatly valued having farm-specific results and recommendations, even if their meningeal worm risk was low, and many adopted prevention methods recommended during the study. All farmers stated that they learned more about the meningeal worm's lifecycle and about factors that elevate risk of meningeal worm infection of animals. Veterinarians also expressed appreciation of this research regarding meningeal worm risk and prevention strategies to share with their clients.

Veterinarians and farmers perceive higher risks to animals or to themselves when they have had personal experience with a specific health concern. Zoonotic pathogen concern differed among veterinarians, with high-risk rankings associated with higher exposure rates due to the nature of their work; lower risk ratings were credited to a healthy immune system and the availability of first-world medical systems. Farmers with no experience of, or no prior knowledge about zoonotic pathogens had little to no concerns about zoonoses. Perceived high risk of anthelmintic resistance was observed in participants who had experience with dewormer-resistant internal parasites in their own livestock or who were aware of how few dewormers remain effective against internal parasites of small ruminants.

All farmers in this study reported having trust in their veterinarian, although their veterinary relationships differed greatly dependent on their level of livestock experience. Farmers with more experience ($\geq$ 25 years) relied on their own knowledge of animal health best practices and would call a veterinary professional only in unusual or extreme cases, for regulatory testing (such as for travel across state borders), or for food safety testing. In contrast,

farmers with fewer years of experience relied more heavily on veterinary visits and phone calls for animal health guidance.

The practices used by farmers in this study use to provide animal health care to animals are often informed by scientific sources in addition to social and peer networking. Farmers were informed primarily by internet searches, veterinarians and books, though many of the seasoned producers attributed their successes to trial-and-error experiences. Reasons producers gave for turning to alternative sources, verses solely to veterinarians, for information included the need to better investigate the broad span of topics their veterinarians briefly mentioned during on-farm visits. Additionally, bonding with other farmers to create a "small farm culture/ community" was highly sought after by farmers. Seeking information via other farmers can help cement mentoring relationships, but farmer-sourced information that is outdated or harmful can lead to poor health outcomes for animals.

Veterinarians perceived two types of cultural practices by farmers in this study that contribute to the success or failure of small ruminant health management. The first entailed responsible and attentive care by producers who have built trust with their veterinarian, thus creating a climate for scientific learning and implementing best practices. The second involved uneducated or misinformed individuals, primarily new, hobby, or economically disadvantaged farmers. Veterinarians perceived farmers who primarily utilize internet searches and social media comments for information on livestock health management as looking for "cheap fixes" to problems that could have been prevented with the well-informed practices. In this study, all veterinarians reported that having no VCPR or having only limited time on farms restricted their ability to teach all the facets of animal health to new farmers.

The need for reliable information for small farmers as to augmentation of veterinarian-derived advice is evident in this research. Challenges to implementation of new animal health strategies were reported by farmers in this study to be largely due to the lack of educational experiences and resources available. Within this context, farmers requested more information about ecological system processes (e.g., watershed effects, wildlife visits, weed growth, etc.). Additionally, farmers sought affordable, on-farm diagnostic tools to inform treatment decisions and to enhance their skills, cut costs, and improve animal health. Farmers sought information about a holistic approach to animal health, and about climate-driven challenges in animal health management. Farmer-veterinarian-service provider collaborative information networks may be solutions to address these needs, perhaps in the form of round tables, digital platforms, listservs, and credential programs, as suggested by the stakeholders in this study. This integration of local/experiential and scientific understanding could comprise a regional animal health system capable of creating more resilient farming communities by generating new tools and ideas which support long-term sustainability.

5.4.1 Future directions

Further research into farmer-veterinarian relationships with the addition of agricultural service providers as liaisons for veterinary health information and local knowledge exchange between the groups could help enhance knowledge of important animal health subjects and communication methods.

5.4.2 Limitations

Given the localized geography and small sample size of our stakeholders (N=10), this study should not be used to generalize about veterinarian-farmer relationships, perspectives, and practices as a whole. Subject-specific sampling ensured a range of descriptions to be included,

but it may have included some bias. As coded words and thoughts were independently selected by one observer, subjective bias may have occurred.

5.4.3 Conclusion

This research explores the complex way that animal health risks are defined, evaluated and acted upon in the context of communities. These patterns of behavior are constantly being modified by lived experiences that includes mentors, social media and veterinary professionals. This holistic perspective toward food animal farming is a necessary foundation for improving veterinarian-client-patient relationships and reducing management firestorms created by misinformation. More educational outreach by agricultural service providers (including academicians), scientifically-informed farmers and veterinarians is needed to improve small ruminant health best practice knowledge and practices, especially with new and hobby farmers.

CHAPTER 6. CONCLUSIONS

Collectively, this work provides the foundation for understanding risk factors and risk reduction methods of *P. tenuis* transmission to small ruminant livestock. This research examined free-living gastropods, and parasites they carry, in pastures grazed by farmed and wild animals in Maine. Though several different species of gastropods were collected, those of the family Succinidea, also called amber snails, were prevalent on six of seven research areas and were the only family to harbor infective stage *P. tenuis*. Specifically, snails with large (14mm+) and medium (9mm to 13mm) sized shells were more likely to contain *P. tenuis* and to carry larval loads considered dangerous to small ruminants. Compared to wild gastropod populations, where an average of 4% are infected with *P. tenuis*, the snails in this study contained an average of 1% and were found on three farms. These wildlife ecology studies gathered prevalence data for wild gastropods often used cardboard traps, which can bias sampling by acting as a pheromone hub, further attracting snails or slugs of a certain species, by accounting for gastropods with burrowing tendencies rather than climbing behavior (such as observed with Succinidea snails), and by allowing more territorial species to dominate. This present study was designed to eliminate sample bias by using stratified random sampling across pastures, but using traps may have gathered more diverse gastropod samples. Interestingly, collected gastropod samples of the genus *Zonitoides* and *Deroceras*, which were reported as prevalent and viable hosts in many other studies, were few and contained no *P. tenuis*. However, some *Deroceras* slugs from this study contained *M. capillaris*.

Gastropods and larval numbers varied across farms, fields, and time in this study. The overwhelming majority of snails came from Farm A and Farm F, both located in mid-coast and with farmers that adopted grazing management practices that was not observed on the other four

farms. At these farms, and at the other research farms with seasonally high abundances of snails, mixed vegetation and broad-leafed plants with tap roots harbored snail populations, even though drought conditions in summer. Snails and slugs were most found in the late spring, and generally became harder to find from July until fall. Conversely, infected snails containing *P. tenuis* were at the highest prevalence in July. These risk factors can inform managers of times in the year to avoid grazing livestock in snail-abundant pastures which contain dandelion, burdock, or nettle. This body of work also found valuable information on controlling high abundance of snails on a pasture-wide scale. Using pastured chickens, which most diversified farms have already on site, abundance of snails can drastically decrease. Furthermore, mowing can also reduce snail populations, though they quickly rebound if the environmental conditions are wet enough and if vegetation is allowed to regrow.

Knowledge, attitudes, and practices around *P. tenuis,* general parasite and health, and veterinarian-client relationships from the perspective of small ruminant farmers and veterinarians was recorded in this study. All participants reported that they knew about meningeal worm, though each differed in the extent of that knowledge or experience, and all reported an increase of knowledge mostly pertaining to the treatment and risk factor research of this study. This chapter was unique in that the participating farmers were part of the two-year risk factor analysis. The farmers (independently) and researchers talked frequently which led to the formation of the survey questions, but also allowed researchers to communicate risk as it was happening. This allowed farmers to make management decisions, such as adding chickens, removing burdock, and avoiding grazing certain areas.

The need for parasite and general animal health best practice education for producers was evident in conversations with farmers and veterinarians. This, and methods for animal health

information gathering, organically came up in conversation when prompted about the challenges veterinarians face when communicating to farmers, and when farmers were prompted to touch upon their sources of information. The farmers in this study reported to use and value their veterinarians for advice as a primary source, however the veterinarians felt that many producers in general, especially new and hobby farmers, routinely use the internet, social media and other farmers to make diagnostic and treatment decisions. Occasionally, this results in the veterinarian having to "clean up" the worsened animal health problem made by misinformed actions of the farmer. The opportunity for animal health and production educators is apparent. Encouraging more roles in this area may help alleviate a partial responsibility in veterinarian visits to new farmers, so that they may focus on larger production goals such as reproduction and product yield.

This work highlights the risk factors of *P. tenuis* on grazing space, provides methods to mitigate snail-borne parasite risk, and provides evidence to encourage consideration of enhanced education efforts, especially on topics of parasite and animal health management.

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APPENDIX A

Table A 1. Digested larval length (μm) measurements.

variable	n	min	max	median	q1	q3	iqr	mad	mean	sd	se	ci
P.tenuis	145	690	1328	1018.00	990.0	1113	123.0	77.095	1050.382	90.442	7.511	14.846
M.capillaris	24	454	735	611.25	555.5	630	74.5	52.484	597.396	67.887	13.857	28.666
Unknown	37	196	1369	550.00	478.0	609	131.0	88.956	570.751	248.126	40.792	82.729

Table A 2. Nested multiplex PCR primer pairs with nanotags and base pair (bp) length of selected genes.

Gene	**Forward Primer**	**Reverse Primer**	**Size (bp)**
18S	18S_1F_Nano	18S_1765R_Nano	1765
	TCTGCGGATGCACTGGTGGAACGGGACAGACACTCGCGCTATATGCTCAGTTAAAAGATTAAGC	GTAAATTCGAGCCTTGGGAGCCCGAGCCGTAGCACCTGATCCTTCTGCAGGTTCACCTAC	
28S-LSU	28S_115F_Nano	LSU_3180R_Nano	3065
	TCTGCGGATGCACTGGTGGAACGGGACAGACACTCGCGCTGAATCTTTCGATGTTAAATCG	GTAAATTCGAGCCTTGGGAGCCCGAGCCGTAGCACCCTTCGCAATGATAGGAAGAGCC	
COI	COI_10F_Nano	COI_848R_Nano	838
	TCTGCGGATGCACTGGTGGAACGGGACAGACACTCGTGGTTTGTGGTCTGGATGGT	GTAAATTCGAGCCTTGGGAGCCCGAGCCGTAGCACCCCGCAGTAAAATAAGCTCGAGAATC	
ITS2	ITS2-F_Nano	ITS2-R_Nano	600
	TCTGCGGATGCACTGGTGGAACGGGACAGACACTCGACGTCTGGTTCAGGGTTGTT	GTAAATTCGAGCCTTGGGAGCCCGAGCCGTAGCACCTTAGTTTCTTTTCCTCCGCT	

Table A 3. Larval identification with associated primer from multiplex.

Snail source ID	Larvae ID	Primers	DNA result	# Pooled Larvae
666	1	ITS2 CO1	*P. tenuis*	
668	1	ITS2	*O. filiformis*	-
785	1	ITS2	*P. tenuis*	
1135	6	ITS2	*M. capillaris*	
1135	8	ITS2	*M. capillaris*	-
1135	12	ITS2	*M. capillaris*	-
1135	15	ITS2	*M. capillaris*	-
1135	16	ITS2	*M. capillaris*	-
1197	1	ITS2 CO1	*P. tenuis*	-
1302	1	ITS2	*Caenorhabditis sp.*	-
1484	1	ITS2	*O. filiformis*	-
1492	1	ITS2	*O. filiformis*	-
1563	1	ITS2 18S	*U. stenocephala*	-
1578	1	ITS2	*Caenorhabditis sp.*	-
1581	1	CO1	*P. tenuis*	-
1581	2	ITS2 CO1	*P. tenuis*	-
1585	1	ITS2	*Caenorhabditis sp.*	-
1588	1	ITS2	*Caenorhabditis sp.*	-
1720	1	ITS2 CO1 18S	*P. tenuis*	-
1720	2	CO1	*P. tenuis*	-
1720	3	ITS2 CO1	*P. tenuis*	-
1720	4	ITS2 CO1 18S	*P. tenuis*	-
1722	1	CO1	*P. tenuis*	-
1722	2	CO1	*P. tenuis*	-
1722	3	ITS2 CO1	*P. tenuis*	-
1885	1	CO1	*P. tenuis*	

Table A3 continued

1885	2	CO1	*P. tenuis*	-
1885	3	CO1	*P. tenuis*	-
1885	4	ITS2 CO1	*P. tenuis*	-
1885	5	ITS2 CO1	*P. tenuis*	-
1885	6	ITS2 CO1	*P. tenuis*	-
1885	7	ITS2 CO1	*P. tenuis*	-
2018	1	ITS2 CO1	*O. filiformis*	-
2039	1	CO1	*P. tenuis*	-
2230	1	CO1	*P. tenuis*	-
2230	2	CO1	*P. tenuis*	-
2230	4	CO1	*P. tenuis*	
2363	2	CO1	*P. tenuis*	-
2511	1	CO1	*P. tenuis*	-
2514	1	ITS2	*Caenorhabditis sp.*	-
2712	1	ITS2 CO1 18S	*P. tenuis*	-
2869	1	ITS2 CO1	*P. tenuis*	-
3063	1	ITS2 CO1	*P. tenuis*	-
3129	1	ITS2	*M. capillaris*	-
3129	2	ITS2	*M. capillaris*	-
3424	1	CO1	*P. tenuis*	
4309	1	CO1	*P. tenuis*	
4223	1	CO1	*P. tenuis*	-
4223	3	CO1	*P. tenuis*	
4223	4	CO1	*P. tenuis*	-
4223	5	CO1	*P. tenuis*	-
4223	6	ITS2 CO1	*P. tenuis*	-
4223	8	CO1	*P. tenuis*	-
4223	10	ITS2 CO1	*P. tenuis*	-

Table A3 continued

4223	11	CO1	*P. tenuis*	-
4223	12	ITS2 CO1	*P. tenuis*	-
4223	13	CO1	*P. tenuis*	
4473	1	CO1	*P. tenuis*	-
4485	1	CO1	*P. tenuis*	-
4485	2	CO1	*P. tenuis*	-
4485	3	CO1	*P. tenuis*	-
4485	4	CO1	*P. tenuis*	-
4499	1	CO1	*P. tenuis*	-
4694	1	CO1	*P. tenuis*	-
4739	1	CO1	*P. tenuis*	2
4779	1	ITS2 CO1	*P. tenuis*	-
4779	2	ITS2 CO1	*P. tenuis*	-
4779	3	CO1	*P. tenuis*	-
4779	4	CO1	*P. tenuis*	-
4779	5	CO1	*P. tenuis*	-
4779	6	CO1	*P. tenuis*	-
4779	7	ITS2 CO1 18S	*P. tenuis* *M. capillaris*	2
4779	8	ITS2	*M. capillaris*	-
4779	9	ITS2 CO1 18S	*P. tenuis* *M. capillaris*	2
4779	10	ITS2 CO1 18S	*P. tenuis* *M. capillaris*	2
4779	11	ITS2 CO1	*P. tenuis* *M. capillaris*	2
4779	12	CO1	*P. tenuis*	-
4779	13	CO1	*P. tenuis*	-
4779	14	ITS2 CO1	*P. tenuis*	-
4779	15	CO1	*P. tenuis*	-
4779	16	CO1	*P. tenuis*	-
4779	18	ITS2 CO1	*P. tenuis*	-

Table A3 continued

4819	1	CO1	*P. tenuis*	-
4856	1	ITS2 CO1	*P. tenuis*	-
4856	2	CO1	*P. tenuis*	-
4856	3	CO1	*P. tenuis*	-
4875	1_3	ITS2 CO1	*P. tenuis* *Crenosoma sp.*	3
4875	4_5	CO2	*P. tenuis*	2
4875	6	CO3	*P. tenuis*	-
4875	7	CO4	*P. tenuis*	-
4875	8	ITS2 CO1	*Crenosoma sp.* *P. tenuis*	2
4875	9	ITS2 CO1	*Crenosoma sp.* *P. tenuis*	2
4875	10	CO7	*P. tenuis*	-
4875	11	ITS2 CO1	*Crenosoma sp.* *P. tenuis*	2
4875	12	ITS2 CO1	*Crenosoma sp.* *P. tenuis*	2
4875	13_14	ITS2 CO1	*Crenosoma sp.* *P. tenuis*	2
4875	15	ITS2 CO1	*Crenosoma sp.* *P. tenuis*	2
4875	16_17	ITS2 CO1	*Crenosoma sp.* *P. tenuis*	2
4876	1	ITS2 CO1 18S	*P.tenuis*	-
4877	1	CO1	*P. tenuis*	-
4877	3	CO1	*P. tenuis*	-
4877	4	CO1	*P. tenuis*	
4877	5	CO1	*P. tenuis*	-

4877	7	CO2	*P. tenuis*	
4877	8	CO1	*P. tenuis*	-
4886	5	ITS2 CO1	*P. tenuis*	-
4886	6	ITS2 CO1	*P. tenuis*	-
4886	13	ITS2 CO1	*P. tenuis*	-
4886	20	CO1	*P. tenuis*	
4886	1_2	ITS2 CO1	*P.tenuis*	2
4886	14_16	ITS2 CO1	*P. tenuis*	2
4886	17_19	CO1	*P. tenuis*	3
4886	21_22	ITS2 CO1	*P. tenuis*	2
4886	23_24	CO1	*P. tenuis*	2
4886	25_26	CO1	*P. tenuis*	2
4886	27_29	ITS2 CO1	*P. tenuis*	2
4886	30_31	ITS2 CO1	*P. tenuis*	
4886	32_33	ITS2 CO1	*P. tenuis*	
4886	3_4	CO1	*P. tenuis*	2
4886	7_8	ITS2 CO1	*P. tenuis*	2
4886	9_12	ITS2 CO1	*Crenosoma sp.* *P. tenuis*	4
4887	1	CO1	*P. tenuis*	-
4992	1	ITS2 CO1	*P. tenuis*	3
5002	1	CO1	*P. tenuis*	2
5066	1	ITS2 CO2	*P. tenuis*	3

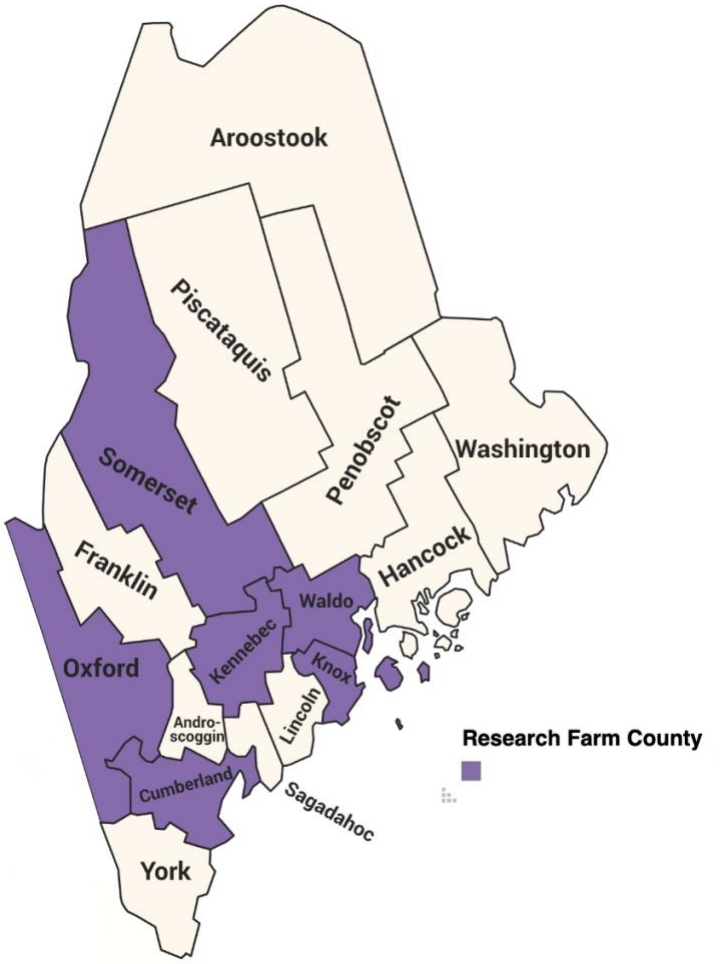

Figure A 1. Map of the state of Maine with counties of study farms highlighted.

Figure A 2. Gastropod sampling schematic. A) Example gridded pasture of 10m x 10m plots, with 12 randomly selected plots for outside (purple squares), verge (yellow squares), and center (blue squares). B) Photo of quadrat in center of pasture.

Table A 4. *P .tenuis* larval load within Succinea classified by shell length.

shell_range	variable	n	min	max	median	iqr	mean	sd	se	ci
Large	P.ten.Dose	9	1	16	2	6	4.222	5.069	1.690	3.896
Medium	P.ten.Dose	36	1	33	2	5	5.250	7.799	1.300	2.639
Small	P.ten.Dose	6	1	2	1	0	1.167	0.408	0.167	0.428

Table A 5. Comparisons of Succinea shell size classification on *P. tenuis* larval loads. Dunn pairwise test with BH p-adjustment showed large differs from small snails ($p<0.01$) and medium differs from small snails ($p<0.01$).

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
P.ten.Dose	Large	Medium	435	3006	-1.775103	0.0758809	0.0758809	ns
P.ten.Dose	Large	Small	435	2074	-3.529178	0.0004169	0.0012506	**
P.ten.Dose	Medium	Small	3006	2074	-3.329893	0.0008688	0.0013032	**

Table A 6. Comparisons of all Succinea (infected and non-infected) snails by pasture location.

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Snail	Center	Outside	2317	1097	-3.6327225	0.0002804	0.0008413	***
Snail	Center	Verge	2317	1959	-0.6371543	0.5240243	0.5240243	ns
Snail	Outside	Verge	1097	1959	3.0119496	0.0025958	0.0038936	**

Table A 7. Comparisons of *P. tenuis*-infected snail by pasture location.

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.sign
P.ten.Pos	Center	Outside	2385	1100	1.3245928	0.1853062	0.2779594	ns
P.ten.Pos	Center	Verge	2385	2034	1.9078270	0.0564136	0.1692407	ns
P.ten.Pos	Outside	Verge	1100	2034	0.2485935	0.8036753	0.8036753	ns

Table A 8. Larval load by pasture location (compared in infected snails).

Site.Place	variable	n	min	max	median	iqr	mean	sd	se	ci
Center	P.ten.Dose	9	1	32	3	9	8.111	10.325	3.442	7.937
Outside	P.ten.Dose	11	1	9	1	3	2.818	2.676	0.807	1.798
Verge	P.ten.Dose	20	1	33	2	6	5.650	7.962	1.780	3.726

Table A 9. Larval load vs. pasture location (compared in all Succinea snails).

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
P.ten.Dose	Center	Outside	2385	1100	1.3213650	0.1863797	0.2795695	ns
P.ten.Dose	Center	Verge	2385	2034	1.9089727	0.0562656	0.1687968	ns
P.ten.Dose	Outside	Verge	1100	2034	0.2526607	0.8005304	0.8005304	ns

Table A 10. Comparisons of all snails by month.

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Snail	05	06	1701	1739	-1.0207620	0.3073672	0.3293220	ns
Snail	05	07	1701	1081	-4.8110507	0.0000015	0.0000225	****
Snail	05	08	1701	635	-3.3284137	0.0008734	0.0026203	**
Snail	05	09	1701	346	1.6656710	0.0957790	0.1105142	ns
Snail	05	10	1701	17	-2.6989350	0.0069562	0.0149061	*
Snail	06	07	1739	1081	-3.9328548	0.0000839	0.0004197	***
Snail	06	08	1739	635	-2.5875887	0.0096650	0.0176270	*
Snail	06	09	1739	346	2.2600947	0.0238154	0.0357231	*
Snail	06	10	1739	17	-2.5563987	0.0105762	0.0176270	*
Snail	07	08	1081	635	0.6469618	0.5176567	0.5176567	ns
Snail	07	09	1081	346	4.6200148	0.0000038	0.0000288	****
Snail	07	10	1081	17	-1.9257304	0.0541380	0.0676726	ns
Snail	08	09	635	346	3.7865667	0.0001527	0.0005728	***
Snail	08	10	635	17	-2.0469654	0.0406615	0.0554475	ns
Snail	09	10	346	17	-3.0435412	0.0023381	0.0058453	**

Table A 11. Comparisons of *P. tenuis*-infected snails by month.

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
P.ten.Pos	05	06	1701	1739	0.8653325	0.3868563	0.5802845	ns
P.ten.Pos	05	07	1701	1081	5.8300570	0.0000000	0.0000001	****
P.ten.Pos	05	08	1701	635	2.3025836	0.0213023	0.0798836	ns
P.ten.Pos	05	09	1701	346	1.6318185	0.1027177	0.2201094	ns
P.ten.Pos	05	10	1701	17	-0.1008190	0.9196941	0.9196941	ns
P.ten.Pos	06	07	1739	1081	5.0930662	0.0000004	0.0000026	****
P.ten.Pos	06	08	1739	635	1.6730142	0.0943245	0.2201094	ns
P.ten.Pos	06	09	1739	346	1.1335435	0.2569861	0.4818489	ns
P.ten.Pos	06	10	1739	17	-0.2219099	0.8243840	0.9196941	ns
P.ten.Pos	07	08	1081	635	-2.3938470	0.0166727	0.0798836	ns
P.ten.Pos	07	09	1081	346	-2.1133024	0.0345749	0.1037247	ns
P.ten.Pos	07	10	1081	17	-1.0282660	0.3038247	0.5063745	ns
P.ten.Pos	08	09	635	346	-0.1622920	0.8710759	0.9196941	ns
P.ten.Pos	08	10	635	17	-0.5357047	0.5921626	0.7834327	ns
P.ten.Pos	09	10	346	17	-0.4863118	0.6267461	0.7834327	ns

Table A 12. Comparisons of *P. tenuis* larval load by month.

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
P.ten.Dose	05	06	1701	1739	0.8660316	0.3864728	0.5797092	ns
P.ten.Dose	05	07	1701	1081	5.8384303	0.0000000	0.0000001	****
P.ten.Dose	05	08	1701	635	2.3128994	0.0207282	0.0777306	ns
P.ten.Dose	05	09	1701	346	1.6273759	0.1036573	0.2221228	ns
P.ten.Dose	05	10	1701	17	-0.1003611	0.9200577	0.9200577	ns
P.ten.Dose	06	07	1739	1081	5.1008597	0.0000003	0.0000025	****
P.ten.Dose	06	08	1739	635	1.6828465	0.0924048	0.2221228	ns
P.ten.Dose	06	09	1739	346	1.1286876	0.2590296	0.4856806	ns
P.ten.Dose	06	10	1739	17	-0.2215497	0.8246644	0.9200577	ns
P.ten.Dose	07	08	1081	635	-2.3907662	0.0168133	0.0777306	ns
P.ten.Dose	07	09	1081	346	-2.1228171	0.0337692	0.1013075	ns
P.ten.Dose	07	10	1081	17	-1.0291418	0.3034130	0.5056884	ns
P.ten.Dose	08	09	635	346	-0.1733924	0.8623430	0.9200577	ns
P.ten.Dose	08	10	635	17	-0.5372026	0.5911277	0.7847659	ns
P.ten.Dose	09	10	346	17	-0.4848078	0.6278127	0.7847659	ns

Table A 13. *P. tenuis* larval load by month.

Month	variable	n	min	max	median	iqr	mean	sd	se	ci
05	P.ten.Dose	1701	0	1	0	0	0.002	0.048	0.001	0.002
06	P.ten.Dose	1739	0	15	0	0	0.016	0.380	0.009	0.018
07	P.ten.Dose	1081	0	33	0	0	0.125	1.352	0.041	0.081
08	P.ten.Dose	635	0	32	0	0	0.099	1.397	0.055	0.109
09	P.ten.Dose	346	0	2	0	0	0.014	0.142	0.008	0.015
10	P.ten.Dose	17	0	0	0	0	0.000	0.000	0.000	0.000

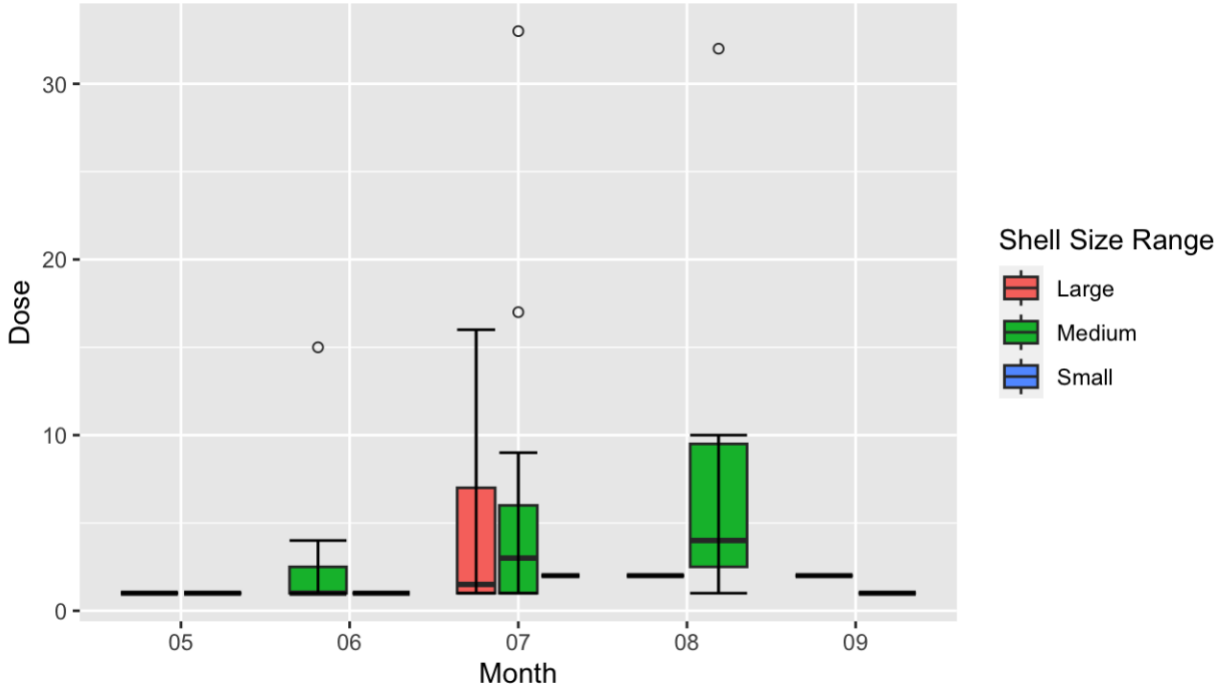

Figure A 3. Larval load and infected snail shell range across months. Circles indicate outliers per month.

Table A 14. First year mow total gastropod abundance by treatment type descriptive statistics. A- mow both years, B- regrowth in second year, C- control/ no mow.

Plot.Section	variable	n	min	max	median	iqr	mean	sd	se	ci
A	Total...Gastropod	96	0	25	0.5	1	1.521	3.506	0.358	0.710
B	Total...Gastropod	95	0	13	0.0	2	1.484	2.449	0.251	0.499
C	Total...Gastropod	94	0	24	2.0	5	3.670	4.549	0.469	0.932

Table A 15. Comparisons of Year 1 gastropod abundance by treatment. A- mow both years, B- regrowth in second year, C- control/ no mow.

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Total...Gastropod	A	B	96	95	0.3690194	0.7121132	0.7121132	ns
Total...Gastropod	A	C	96	94	4.4235303	0.0000097	0.0000291	****
Total...Gastropod	B	C	95	94	4.0449745	0.0000523	0.0000785	****

Table A 16. Comparisons of blocking effect on the first year of mowing.

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Total...Gastropod	1	2	71	73	1.3005371	0.1934170	0.2901254	ns
Total...Gastropod	1	3	71	71	3.7710405	0.0001626	0.0009754	***
Total...Gastropod	1	4	71	70	1.0666621	0.2861245	0.3433494	ns
Total...Gastropod	2	3	73	71	2.4966009	0.0125390	0.0250780	*
Total...Gastropod	2	4	73	70	-0.2218622	0.8244211	0.8244211	ns
Total...Gastropod	3	4	71	70	-2.6909822	0.0071242	0.0213726	*

Table A 17. Second year total gastropod abundance by treatment type. A- mow both years, B- regrowth in second year, C- control/ no mow.

Plot.Section	variable	n	min	max	median	iqr	mean	sd	se	ci
A	Total...Gastropod	144	0	44	0	7.0	5.104	8.931	0.744	1.471
B	Total...Gastropod	144	0	110	19	28.5	24.000	22.089	1.841	3.639
C	Total...Gastropod	132	0	132	25	57.0	38.455	36.559	3.182	6.295

Table A 18. Comparisons of Year 2 gastropod abundance by treatment. A- mow both years, B- regrowth in second year, C- control/ no mow.

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Total...Gastropod	A	B	144	144	8.384748	0.0000000	0.0000000	****
Total...Gastropod	A	C	144	132	10.518077	0.0000000	0.0000000	****
Total...Gastropod	B	C	144	132	2.317632	0.0204693	0.0204693	*

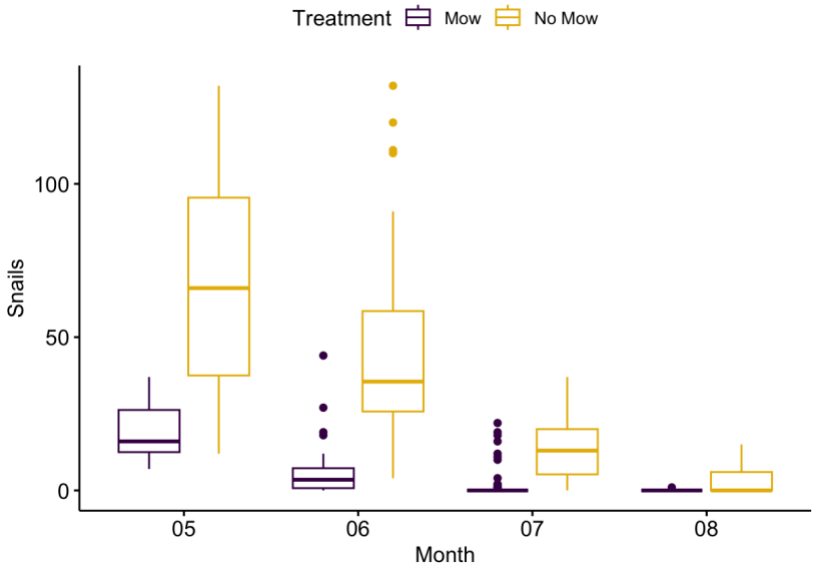

Figure A 4. Year 2 treatments by month. Circles indicate outliers.

Table A 19. Year two treatment descriptive statistics by month.

Treatment	Month	mean	median	SD
Mow	05	19.0416667	16.0	9.2899158
Mow	06	5.6250000	3.5	7.9080487
Mow	07	2.0517241	0.0	5.2529771
Mow	08	0.0416667	0.0	0.2041241
No Mow	05	67.5000000	66.0	31.8194560
No Mow	06	44.0543478	35.5	26.3302626
No Mow	07	13.4268293	13.0	9.9542540
No Mow	08	3.5000000	0.0	4.7877158

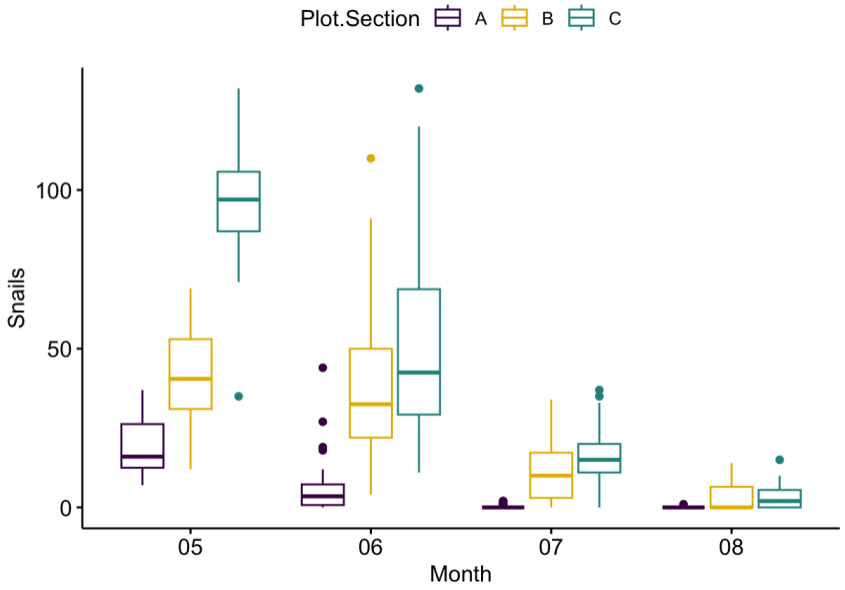

Figure A 5. Year 2 plots by month. A- mow both years, B- regrowth in second year, C- control/ no mow. Circles indicate outliers.

Table A 20. Blocking effects on soil moisture in year two of mow treatment.

.y.	group1	group2	n1	n2	statistic	p	p.adj	p.adj.signif
Soil.moisture	1	2	108	108	-0.8801706	0.3787669	0.3787669	ns
Soil.moisture	1	3	108	108	-2.4324248	0.0149981	0.0316741	*
Soil.moisture	1	4	108	96	-3.2665399	0.0010887	0.0065322	**
Soil.moisture	2	3	108	108	-1.5522542	0.1206014	0.1809021	ns
Soil.moisture	2	4	108	96	-2.4126490	0.0158371	0.0316741	*
Soil.moisture	3	4	108	96	-0.9067412	0.3645436	0.3787669	ns

APPENDIX B

Farmer interview prompt:

Thank you for participating in the Stakeholder Knowledge, Attitudes and Practices: Parasite Risk Management for Small Ruminant Health study. The purpose of the research is to gather stakeholder knowledge, attitudes and practices of parasite (e.g., meningeal worm) transmission and animal health risk factors, in addition to control strategies. You must be 18 years of age or older to participate. By participating, you agree to have your answers recorded by audio or written methods. Do you agree to have your audio recorded? This interview will last about one hour. If you wish to not answer any question, please indicate so, and we will skip to the next question. Your identifying information, including farm name, will remain confidential, and an identifier, such as "Farmer A," will be associated with your responses and data. Do you have any questions before we get started?

Farmer Interview Questions:

1) How long have you been farming?
2) How did you get interested in farming?
3) How hands-on are you with your livestock?
4) Describe your grazing management/ feeding regime for your livestock (e.g., rotational, supplemental feed, seasonal changes, etc.)
5) What is your animal health husbandry like?

a) Do you have an annual health schedule (vaccinating, deworming, shearing, etc.)?

b) How do you make decisions in regard to routine animal health husbandry (individual animal care, production goals, time, costs, etc.)

c) How involved are veterinarians at your farm?

6) Do you have any past or current animal health concerns relating to parasites?

a) What are they?

b) What treatment/ control methods did you use?

c) What have these issues cost you (financially, emotionally, etc.)?

7) What was your perceived risk of meningeal worm before this project?

a) Has this changed based on the information that I've shared?

8) Have you experienced a change in your knowledge with regard to meningeal worm?

a) Why/ why not?

9) Have you experienced a change in your behavior/ management with regard to meningeal worm?

a) Why/ why not?

b) Specifically, what changes have you made in your management to reduce parasites/ animal infections?

10) Based on my recommended management strategies for gastropods/ meningeal worm, will you consider them in your system? Are you considering using some, or all, of my management strategies for gastropods/meningeal worm on your farm?

a) If not, why not? Can you identify barriers (cost, time, etc.)

i) If time/ cost (or other given barrier) wasn't a factor would you do this. What would make it possible to help you do this.

b) If so, to what extent/ how will you go about implementing recommendations on your farm?

11) What are your sources for animal health information?

12) On a scale of 1 to 3, how concerned are you about anthelmintic resistance?

1 being not at all concerned, 2 being somewhat concerned, 3 being very concerned.

a) Why/ why not?

13) On a scale of 1 to 3, how concerned are you about zoonotic pathogens/ parasites?

1 being not at all concerned, 2 being somewhat concerned, 3 being very concerned.

b) Why/ why not?

14) Is there anything else you would like to share with me regarding animal health and parasites?

APPENDIX C

Veterinarian interview prompt:

Thank you for participating in the Stakeholder Knowledge, Attitudes and Practices: Parasite Risk Management for Small Ruminant Health study. The purpose of the research is to gather stakeholder knowledge, attitudes and practices of parasite (e.g., meningeal worm) transmission and animal health risk factors, in addition to control strategies. You must be 18 years of age or older to participate. By participating, you agree to have your answers recorded by audio or written methods. Do you agree to have your audio recorded? This interview will last about one hour. If you wish to not answer any question, please indicate so, and we will skip to the next question. Your identifying information, including practice name, will remain confidential, and an identifier, such as "Veterinarian A," will be associated with your responses and data. Do you have any questions before we get started?

Veterinarian Interview Questions:

1. How long have you been a veterinarian?
2. Do you own livestock or farm?
3. How often are the instances of parasitic- related illnesses you have observed or treated, with 1 being not common, 2 being somewhat common, 3 being very common?
4. Have you experienced any presumed meningeal worm cases?
    a. How often?

b. How do you diagnose?

c. Any patterns, like time of year?

d. What species had it/ were most impacted?

5. Can you describe the meningeal worm transmission cycle?

a. What are the risk factors?

6. What do you recommend to farmers for control/ treatment methods for parasites in general? For meningeal worm?

a. Does this include pasture management? Vector management?

7. Based on my recommended management strategies for gastropods/ meningeal worm, will you consider sharing them to farmers?

a. Why/ why not?

8. What would help you in your decision making/ communications to farmers with regard to parasites in general and to meningeal worm, specifically?

9. How often do you bring up human health risks of zoonotic pathogens/ parasites to your clients?

10. On a scale of 1 to 3, how concerned are you for anthelmintic resistance? 1 being not at all concerned, 2 being somewhat concerned, 3 being very concerned.

a. Why/ why not?

11. On a scale of 1 to 3, how concerned are you for zoonotic pathogens/ parasites?

1 being not at all concerned, 2 being somewhat concerned, 3 being very concerned.

a. Why/ why not?

12) Is there anything else you would like to share with me regarding animal health and parasites?

BIOGRAPHY OF THE AUTHOR

Rachel White was born in Oakland, Maine on February 4th, 1992. She lived in the Belgrade Lakes region during her childhood and graduated from Messalonskee High School in May 2010. Upon graduation, she attended Unity College, in Unity Maine. During this time, she also ran an equestrian business, giving riding lessons and horse camps, as well as serve as the Program Director for a local lake organization. She finished her B.S. degree in Biology in 2014 and continued with her position as a Program Director. In 2016, she became a high school science teacher at Maine Arts Academy. In 2019, Rachel joined Dr. Anne Lichtenwalner's lab and in 2020, she joined the One Health NRT. She was also a teaching assistant for Chemistry and Ecology and Environmental Sciences, as well as adjunct for Animal Science. Rachel is a candidate for the Doctor of Philosophy degree in Ecology and Environmental Sciences from the University of Maine in August 2024.

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