

**Determining the Prevalence of the Raccoon Roundworm (*Baylisascaris procyonis*) in
New Brunswick, Canada**

By

Riley Arthur Oremush

A thesis submitted to the Department of Biology
Mount Allison University
in partial fulfillment of the requirements for the
Bachelor of Science degree
With Honours in Biology

April 2022

Table of Contents

<i>Abstract</i>	3
<i>Section 1: Introduction</i>	4
1.1 - Parasitism & Zoonoses	4
1.2 - Risk Factors of Zoonoses.....	5
1.3 - Raccoons	5
1.4 - The Raccoon Roundworm.....	6
1.5 - Raccoon Roundworm Lifecycle	9
1.6 - Baylisascariasis.....	11
1.7 - The Raccoon Roundworm & Climate Change	12
<i>Section 2: Materials and Methods</i>	14
2.1 - Raccoon Latrine Identification	14
2.2 - Wildlife Population Estimation.....	14
2.3 - Raccoon Roundworm Egg Isolation	15
2.4 - <i>B. procyonis</i> Egg Identification	16
2.5 Roundworm DNA-Extraction	16
2.6 - PCR amplification of <i>B. procyonis</i> DNA.....	17
2.7 - Data Analysis.....	19
<i>Section 3: Results</i>	20
3.1 Raccoon Latrine Identification & Wildlife Population Estimation.....	20
3.2 Ecological Population Determination	20
3.3 <i>B. procyonis</i> Egg Identification, Quantification & Confirmation.....	20
3.4 Risk Factor Modeling of <i>B. procyonis</i> Prevalence	22
<i>Section 4: Discussion</i>	28
4.1 Identification of <i>B. procyonis</i> in New Brunswick, Canada	28
4.2 Effect of Ecological & Environmental factors on <i>B. procyonis</i> egg prevalence	29
4.3 Future Directions.....	30
4.4 Conclusion	31
<i>Bibliography</i>	32
<i>Appendix:</i>	42

Abstract

The raccoon roundworm (*Baylisascaris procyonis*) is an intestinal nematode mainly found within its primary definitive host of raccoons (*Procyon lotor*). Contraction of the raccoon roundworm by humans can cause the development of Baylisascariasis, a potentially deadly disease that can also cause blindness and a variety of other negative health effects. With the largest risk group consisting of adolescents, and the lack of extensive research on the parasite within the province of New Brunswick, further understanding of the raccoon roundworm and the ecological factors influencing its prevalence is of great epidemiological importance. This study sampled feces from 8 raccoon latrines across the province to determine the prevalence of the raccoon roundworm in New Brunswick for the first time in 10 years. These results were correlated to ecological factors including sightings of domestic animals and of wildlife (excluding raccoons) determined by the establishment of night-vision camera traps across 28 locations around the region of south-eastern New Brunswick for 4-months in 2021. Fecal floatation was performed to isolate potential roundworm eggs for subsequent microscopy and DNA analyses of samples. Raccoon roundworm eggs were microscopically identified across 6 latrine locations, while 3 locations were molecularly confirmed via PCR and genetic sequencing. Fecal samples from latrines with lower roundworm EPG counts ranged from 0 – 8.3, and would be undetectable by PCR analysis, while latrines with higher roundworm EPG counts range from 76.2 – 159.6 (overall median = 5.40 EPG, IQR = 17.6). Increased incidence of *Baylisascaris procyonis* positive latrine samples were correlated to increased sightings of domestic animals, which may present a possible source of greater risk to humans inhabiting regions with this factor.

Section 1: Introduction

1.1 - Parasitism & Zoonoses

Inherent to the survival of life is the requirement to source energy. Over the course of their evolutionary history, organisms have adapted to survive through the use of a variety of strategies. One example of these strategies is parasitism. Parasitism can be defined as the “association between two different organisms wherein one benefits at the expense of the other” (Olano *et al.*, 2011). With more than half of all life on Earth engaging in parasitic interactions, the diversity of organismal groupings is vast, with only a small fraction that affects *Homo sapiens* (Lutermann, 2019). Nevertheless, parasitism has often been the cause of many illnesses, which have plagued humankind, historically and currently causing a wide variety of diseases to humans, many of which are fatal. Our ability to combat some of these parasitic organisms has recently evolved. While parasites’ decrease a host’s fitness, parasitism does present ecological value by ensuring a wide range of genetic diversity among individuals within a species. This process can positively influence the demographics of an ecosystem in fortifying its survivability and success (Preston & Johnson, 2010). Thus, parasites and parasitic behaviour are major influences to life on earth, including humans.

Parasitic organisms or microbes from animal hosts that cross the species barrier to affect humans are known as zoonotic parasites or pathogens, respectively, and cause zoonoses – novel animal-sourced diseases in humans (Barnes *et al.*, 2017). Zoonoses are one of the major causes of disease among humans, with approximately 75% of newly identified diseases being of zoonotic origin (Bidaisee & Macpherson, 2014; Woolhouse *et al.*, 2001). Zoonotic parasites and pathogens have a tendency to transition across a variety of animal species and continue to evolve as they specialise to new host organisms (Cleaveland *et al.*, 2001). Modern significant examples of this can be seen with SARS-CoV-2, the virus which causes the COVID-19 disease; Ebola virus; Influenza; as well as Lyme disease, all having been sourced to have begun propagation through zoonotic based transmission (Christou, 2011).

1.2 - Risk Factors of Zoonoses

Successfully combatting the propagation of zoonotic diseases can be achieved through increasing our understanding of factors influencing their prevalence (Bidaisee & Macpherson, 2014). Researching environmental, socioeconomic and demographic factors are especially relevant among developing countries such as those in the region of South-East Asia, where 90% of mortalities caused by new zoonotic-associated diseases are considered avoidable (World Health Organization, 2005). Another major factor influencing zoonotic-based disease propagation from particular animals is their proximity to humans (Cleaveland *et al.*, 2001; Dobson & Foufopoulos, 2001; Woolhouse *et al.*, 2001). The rate of zoonotic pathogens crossing over to human populations is highly sensitive to interactions between humans and wildlife (Pappaioanou *et al.*, 2009). Animals with ecological and geographical niches distinct from humans or timid animals with low rates of interactions are less likely to be the origins of zoonotic-based diseases affecting humans, while more human-tolerant animals such as some birds and rodents often interact with humans in urban spaces. Such species have a higher potential to transmit pathogens or parasites (Pappaioanou *et al.*, 2009). A greater understanding of zoonoses requires further biological comprehension of these systems of exposure in addition to other risk factors to human populations.

1.3 - Raccoons

Raccoons (*Procyon lotor*) are omnivorous mammals found widespread across the majority of North America, with wildlife habitats generally ranging from wetlands, plains, and forests with nearby water-sources (Chow *et al.*, 2005). These animals are highly adaptable and are often found within urban areas, in close proximity to humans (Chow *et al.*, 2005). Native to North America, the genus of *Procyon* consists of three nocturnal raccoon species, the most prolific being the Common Raccoon (*P. lotor*), simply referred to as raccoons. These animals diverged evolutionarily from the closest related extant genus of *Bassariscus* (which include ring-tailed cats and cacomistles) roughly 10 million years ago (Helgen *et al.*, 2013). As with the majority of American fauna, raccoons experienced diversification 2.7 million years ago during the Great American Biological Interchange between North and South America, leading to their current distribution within the Americas (O'Dea *et al.*, 2016). Raccoons are also an invasive species to parts of both Europe and Asia, initially introduced by humans with the popularity of the fur trade in the early 20th century, their population in central Europe has tripled since 1990 (Salgado, 2018).

Raccoons are increasingly common in urbanized settings, with their opportunistic, omnivorous scavenging behaviour incredibly well-suited to human-dense regions (Prange *et al.*, 2004). While no biological predators are found in highly urban areas, a form of non-specific predation still occurs on raccoon populations, by fast-moving vehicles (Prange *et al.*, 2003). Their lack of natural predators, coupled with excess food availability in these areas contributes to strong growth in raccoon populations (Riley *et al.*, 1998). Raccoons within urban areas are generally tolerated by humans and do not pose a direct threat. However, they have been found to be carriers of parasites and zoonotic pathogens. They are potential transmitters of rabies and leptospirosis, and are the definitive host for the emerging zoonotic parasite, the racoon roundworm (*Baylisascaris procyonis*) (Kazacos, 2016; Riley *et al.*, 1998).

1.4 - The Raccoon Roundworm

Baylisascaris procyonis (*B. procyonis*) is an intestinal roundworm mainly found in their primary definitive host animal of racoons (Sprent, 1968). Natural propagation of *B. procyonis* occurs through a definitive host's ingestion of infective roundworm eggs, generally found within a previous host's feces, often raccoons (Kazacos, 2001). The propagation of *B. procyonis* relies on their ability to be ingested by definitive hosts; the likelihood of this event is enhanced by the communal defecation by raccoons within a family, within a central, known as a "raccoon latrine" (Page *et al.*, 1998). Raccoon latrines are often within or close to their original habitat and as such provide ideal locations for roundworm egg ingestion by fellow raccoons, likely being the most common setting of contraction (Thornton *et al.*, 2020). While not harmful to their definitive host, *B. procyonis* eggs have the potential to cause a great deal of harm if ingested by non-definitive hosts, known as paratenic, or alternative hosts (Graeff-Teixeira *et al.*, 2016). Paratenic hosts of *B. procyonis* commonly include many small mammals, some bird species and notably, humans (Graeff-Teixeira *et al.*, 2016). The number of humans coming into contact with raccoon latrines has grown with an increase in urban and suburban areas (French *et al.*, 2019). Once ingested by a paratenic host, newly hatched larvae will migrate to various regions of the body and feed on these locations (Gavin *et al.*, 2005). As *B. procyonis* larvae continue to growth within their paratenic hosts, they can cause a variety of negative health effects (Gavin *et al.*, 2005).

While raccoons are the primary definitive host for *B. procyonis*, other species, including dogs and skunks can also serve as "alternative definitive hosts" (Kazacos, 2001). While dogs can still

function as alternative definitive hosts, their infective egg output as well as duration of time between initial infection and subsequent shedding of eggs, are inferior to their raccoon counterparts (S. G. H. Sapp *et al.*, 2020). Nevertheless, the roundworms' ability to continue to propagate even outside their primary host species makes their threat level to humans much higher (Page, 2013). While individuals may not come into contact with raccoons and their feces, interaction with pet dogs and their feces is more common (Page, 2013).

Prior to its classification by Sprent in 1968, species under the genus of *Baylisascaris* were originally grouped within nematodes of genera *Ascaris* or *Toxacara* (Sprent, 1968). *Baylisascaris* species have since become reorganized based on the presence of cervical alae with cuticle bars reaching the surface of the cuticle, with an additional characteristic of male *Baylisascarids* based on roughened pericloacal areas (Kazacos, 2001; Sprent, 1968). Morphological analyses have currently determined 10 *Baylisascaris* species, each of which associated with a different definitive host (S. G. H. Sapp *et al.*, 2017). With the exception of *B. laevis*, *Baylisascaris* hosts are all carnivorous mammals (S. G. H. Sapp *et al.*, 2017). Four of these nematode species have had their complete mitochondrial genome researched, with phylogenetic relationships currently identifiable (Fig. 1.1) (Xie *et al.*, 2011). Compared to their more closely related species, *B. columnaris* (skunk roundworm), the raccoon roundworm is more dangerous as they require fewer infective eggs ingested as well as fewer maturing larvae in the brain to cause equivalent negative symptoms (Kazacos, 2016). This has been found to be the result of the larvae continuing to molt and grow as they migrate within the host (Dangoudoubiyam & Kazacos, 2009). An additional contributing factor to the increased threat level imposed by *B. procyonis* is due to the increased prevalence of the parasites' respective definitive hosts with relation to human dense regions (Broadfoot *et al.*, 2001). While skunks are also commonly found within human populations, with estimated metapopulations in urban areas being near 6.4 to 12.6 animals/km², raccoon populations are considerably greater, possessing estimated metapopulations of 37 to 94 animals/km² (Broadfoot *et al.*, 2001).

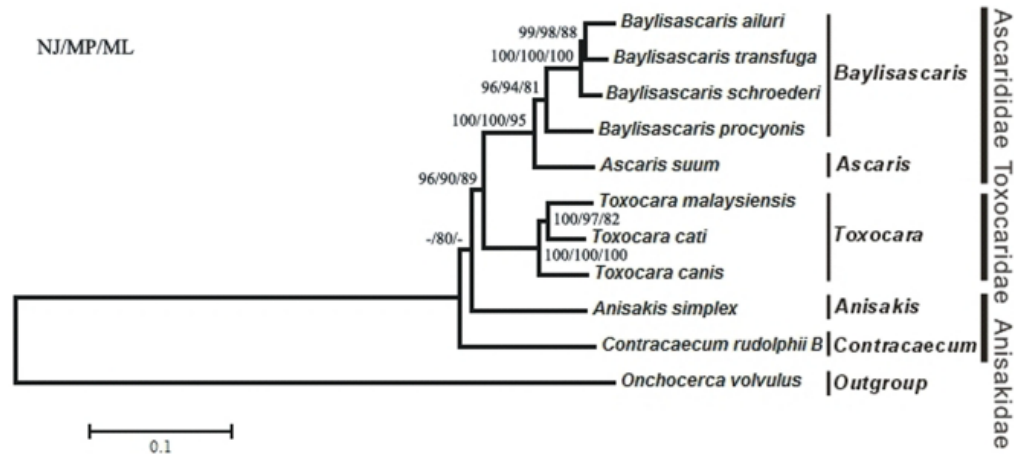


Figure 1.1. Phylogenetic relationships of ten Ascaridida species for which complete mitochondrial DNA (mtDNA's) are available, inferred from NJ, MP and ML analysis for amino acid sequence data derived from 12 protein coding genes, using one filarioid species (*O. volvulus*) as the outgroup. Figure from (Xie *et al.*, 2011).

Ascarid (roundworm) eggs generally consist of three major layers; an outer, uterine layer, a middle chitinous layer, and a proteinaceous coat surrounding the ascarid egg known as the lipid layer (Gavin *et al.*, 2005; Kazacos & Turek, 2011; Roussere *et al.*, 2003). *B. procyonis* eggs are very similar to fellow members of the family Ascarididae; measuring from 65-80 μ m by 55-60 μ m (Graeff-Teixeira *et al.*, 2016; Piero *et al.*, 2012). The brown-coloured *B. procyonis* eggs possess a finely-granular surface in comparison to fellow Ascarids, such as the dog roundworm (*T. canis*), which are larger and more coarse (Kazacos, 2016; Piero *et al.*, 2012). Raccoon roundworm eggs exhibit slight changes in morphology according to their level of development (Kazacos, 2016; Piero *et al.*, 2012). Beginning in its unfertilized stage, the eggs appear slightly “ellipsoidal” in shape and are largely dominated by the embryo within (Piero *et al.*, 2012). Once fertilized, the eggs take on a more rounded shape as the larvae begin to develop and reach their infective life-stage, with the parasite eventually becoming observable from within the eggs (Gavin *et al.*, 2005). Once hatched, the racoon roundworm larvae and adult life stages become more similar morphologically to the dog roundworm, *Toxocara canis* (*T. canis*), with a tan-white colour, cylindrical in shape and tapered at both ends (Despommier, 2003). The presence of large, paired lateral alae enable along with their centrally located intestine enable initial identification of *Baylisascaridae* larvae (Graeff-Teixeira *et al.*, 2016). An additional distinguishing character includes a much larger maximum diameter at the larval stage, measuring 60 to 70 μ m; while closely

related Ascarids are considerably smaller; *T. canis* measures 14 to 20µm, *T. leonina* measures 25-28µm, while *A. lumbricoides* measures 35 to 45µm in diameter (Graeff-Teixeira *et al.*, 2016).

1.5 - Raccoon Roundworm Lifecycle

The lifecycle of *B. procyonis* begins as an “unembryonated” egg, which enters the environment through travel within definitive host feces (Lee *et al.*, 2010). In the following two-to-four weeks, the embryos develop to the stage known as “embryonated”; at which point eggs have become infective (Lee *et al.*, 2010). The direct life-cycle of *B. procyonis* is a definitive host – definitive host process, based largely on their contraction by adolescent raccoons via ingestion of infective roundworm eggs (S. G. H. Sapp *et al.*, 2017). In contrast, adult raccoons and dogs have been found to primarily contract the roundworm via third-stage larvae through ingestion of a paratenic host (Sapp *et al.*, 2020). Infective roundworm eggs ingested by a definitive host travel to the small intestine, wherein the larvae hatch. Within the small intestine of a definitive host, these roundworm larvae are non-harmful and eventually mature to egg-laying adults, where propagation repeats following host defecation containing the eggs (Lee *et al.*, 2010). Female roundworms are capable of laying an estimated 115,000 to 877,000 eggs per day, while the raccoons can shed up to 45,000,000 eggs per day (Hamann *et al.*, 1989; Jacobson *et al.*, 1982; Kazacos & Boyce, 1989; Sorvillo *et al.*, 2002).

Paratenic, or intermediate hosts of *B. procyonis* include a wide variety of animals such as humans, rats and various species of birds and contract *B. procyonis* through accidental ingestion of infective eggs (Lee *et al.*, 2010). Similar to their direct lifecycle, infective *B. procyonis* eggs within a paratenic host descend to the intestinal tract and subsequently hatch (Kazacos, 2001). However, unique to paratenic hosts, recently hatched roundworm larvae will often migrate to various regions within the organs, becoming encapsulated in granulomas in a process known as “somatic migration” (Kazacos, 2001). The roundworm larvae will remain at this life-stage within these organs and tissues until the paratenic host is predated, and the larvae is ingested by a definitive host, where they can then progress to their adult and reproductive stage (Kazacos, 2001; Lee *et al.*, 2010). Somatic migration can be very harmful, depending on their location of migration. This process of definitive host – paratenic host – definitive host differs from the roundworm’s direct lifecycle and is aptly known as its “indirect lifecycle” (Fig. 1.2).

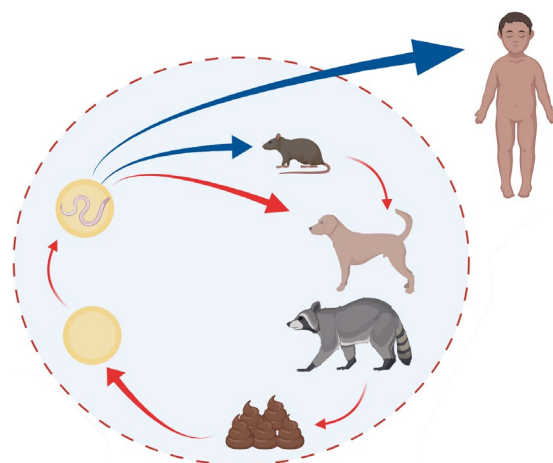


Figure 1.2. Visualization of *B. procyonis* direct and indirect lifecycle. Arrows shown in red indicate the direct lifecycle of *B. procyonis*, traveling from definitive host to definitive host, while arrows in blue indicate the indirect lifecycle of *B. procyonis*, with the presence of an intermediate, paratenic host. Note that humans are not traditionally predated upon by definitive hosts of *B. procyonis*, however still exhibit the associated negative health-effects, thus are visualized outside either lifecycle. Figure adapted from (Bauer, 2012).

Within their definitive hosts, *B. procyonis* resides in the small-intestine and is non-harmful, as this would likely place the hosts' ability to successfully propagate eggs at risk (Ewald, 1995). However, within paratenic hosts; including rats, humans and various species of birds, the roundworm larvae will migrate across the infected host; likely as a means to increase the odds of being consumed by definitive hosts and not solely being found within the small intestine (S. G. H. Sapp *et al.*, 2017). The largest factor of successful propagation and continuance of *B. procyonis* is due to the increased tolerance and resistance of infective eggs prior to ingestion while still within the external environment (Kazacos, 2001). The viability of raccoon roundworm eggs has been tested under various conditions, with the loss of infective capabilities observed once temperatures reach upwards of 62 degrees Celsius, while their lower temperature parameters still unknown, as eggs were found still viable while in conditions under 15 degrees Celsius for 6 months (Shafir *et al.*, 2011). Furthermore, the eggs have also been found to remain functional under increasingly hardy conditions; with treatment of 20% bleach causing the eggs to lose their sticky, outer proteinaceous coat (Roussere *et al.*, 2003), however retaining viability. Roundworm eggs have also been found to remain viable following treatment of pure, 100% bleach (Shafir *et al.*, 2011).

As previously mentioned, parasites are incredibly ecologically diverse, possessing a variety of unique strategies to successfully siphon host-animal resources. These strategies can be broadly grouped by the means by which a parasite resides within a host; ectoparasites include a variety of mainly mobile organisms and are defined by their living outside of a host animal, notable examples include ticks and more broadly – mosquitos. In contrast, endoparasites include organisms which reside within the host body, with examples varying from parasitic nematodes and trematodes (Holand *et al.*, 2015; Lehmann, 1993). Further demarcations of parasites come in the analysis of their life-history trade-off's via the degree to which a parasite siphon's resources from their host animal, before limiting its own respective fitness. *B. procyonis* is understood to be an endoparasite exhibiting predator-borne transmission to a definitive host animal (Ewald, 1995). This form of transmission is defined by a definitive host animal predating upon an infected intermediate host and can be seen in the raccoon roundworm via adult raccoon predation of infected intermediate hosts.

1.6 - Baylisascariasis

Within humans, infection of *B. procyonis* larvae is known as, “baylisascariasis”, a zoonotic disease that is represented by a wide variety symptoms (Gavin *et al.*, 2005; Sorvillo *et al.*, 2002). While baylisascariasis is the disease associated with human contraction of *B. procyonis*, the ailments associated with roundworm larval infection are incredibly diverse and are grouped under the term, larva migrans (LM) (Fox *et al.*, 2010). LM consists of the migration of newly hatched larvae to various tissues or organs within the body. LM often has an additional prefix, depending on the location of larval migration, many of which have unique associated-symptoms and outcomes. The most common form of LM is known as visceral larva migrans (VLM) and is characterized by the nematode larvae migrating to the body's major organs (Despommier, 2003). Symptoms associated with VLM include necrosis of the liver, enlarged spleen as well as respiratory problems, depending on the targeted organ(s) (Despommier, 2003). Ocular larva migrans (OLM) includes migration within the body to specifically include the eye and its associated structures and can lead to blindness. Cutaneous larva migrans (CLM) is defined by the migration of roundworm larvae to the cutaneous layer and is associated with cases involving skin eruptions (Yoshida *et al.*, 2016). Finally, the location of greatest potential danger are those associated with neural larva migrans (NLM), which have been found to often prove fatal or deal

permanent neurological damage to the individual as the roundworm larvae migrate to the brain or spinal cord (Yoshida *et al.*, 2016). Treatment of LM is often time-sensitive, due to the development and onset of symptoms prior to the seeking of medical assistance (Perlman *et al.*, 2010).

Raccoon latrine sampling is one of the three major methods utilized to quantify the prevalence of *B. procyonis* which additionally include necropsy and fecal analysis of live-trapped raccoons (Page *et al.*, 2005). These testing methods are done based on desired results, each with varying degrees of effectiveness (Smyser *et al.*, 2010); necropsy has been found to be the superior method of testing in terms of explaining the most variance, however, is also laborious and time-consuming. As a means of reducing labor and time, this study utilized latrine sampling due to its increased ease and as it is the major means to determine the threat level of the raccoon roundworm on human populations (Smyser *et al.*, 2010). While this method is unable to be used to determine *B. procyonis* prevalence within individual raccoons due to the inherent homogenized nature of latrine sampling, this extraction method enables the non-invasive determination of risk imposed on human populations.

Wildlife rehabilitators, as well as individuals in close contact with raccoons are at a greater risk of *B. procyonis* contraction (S. Sapp *et al.*, 2016). Thus, when handling potentially contaminated fecal samples in the environment, proper PPE is worn in the form of masks, latex gloves, in addition to a hazmat suit to ensure a reduced risk of contraction.

1.7 - The Raccoon Roundworm & Climate Change

With the continued consumption of fossil fuels, global climate change poses a large impact on wildlife populations across the world, leading many animals to extinction (Hof *et al.*, 2012). It has been further hypothesized that hardy, generalist animals such as rats, cockroaches and raccoons will be able to survive these environmental effects (Hof *et al.*, 2012). Many of these animals utilize anthropogenic food sources as a means to potentially compensate for the lack of natural resources (Bozek *et al.*, 2007). With the increase in range of urban and suburban regions over time in tandem with increasing raccoon densities within these landscapes; it is possible that the rate of contraction of the *B. procyonis* by humans will likewise increase over time, leading to a higher risk of the roundworm-associated disease of Baylisascariasis (Gordon *et al.*, 2016).

As biodiversity decreases within an environment, it's been hypothesized that there is a resultant increase in the risk of disease contraction among the remaining wildlife (Ferraguti *et al.*, 2021).

This process, known as the dilution effect hypothesis, has been theorized to be a major factor influencing the potential for disease transmission among wildlife within urban and suburban regions (Civitello *et al.*, 2015). With an increase in urbanization, in addition to a reduction in overall biodiversity as a result of climate change, the risk of contraction of Baylisascariasis is of increasing epidemiological importance.

B. procyonis has been confirmed within humans in the countries of Canada, USA and Germany, with the majority being reported in USA; as of 2012, there have been 4 confirmed cases, 23 reported since 1973 and 21 since 1993, demonstrating an increase in the disease (Graeff-Teixeira *et al.*, 2016). The rate of infection across various demographics shows the greatest incidence of contraction found within young male children, while a second demographic factor of note includes a spike in infections among adults aged greater than 50 (Graeff-Teixeira *et al.*, 2016). Despite these reported cases, recent studies have suggested a lack of testing being a possible factor leading to a reduced representation of the disease within populations (Gavin *et al.*, 2005). Examples of this can be seen with the positive diagnosis of *B. procyonis* found following brain scans in an autopsied 73 year-old deceased Alzheimer's patient; it was hypothesized the Alzheimer's condition had masked the symptoms of the Baylisascariasis disease (Graeff-Teixeira *et al.*, 2016). Major risk factors associated with contraction of *B. procyonis* include contact with raccoon latrines, ingestion of dirt, known as pica contaminated by dog, cat or raccoon feces, as well as the male sex (Strausbaugh *et al.*, 2004).

Ultimately, the raccoon roundworm is a recently discovered deadly parasitic nematode, with little overall information on its presence within the Maritime provinces. With the hardiness of *B. procyonis* eggs demonstrated, and their potential to cause severe harm to human populations; it is recommended that if raccoon fecal matter is identified on property, decontamination of the biohazardous material should/must proceed. Little governmental information can be found within the Maritime provinces; this study serves to provide an initial insight into roundworm population in these regions, specifically within rural regions, which make up slightly less than half the human population. This study analyzed the prevalence of the raccoon roundworm in Maritime Canada for the first time in 10 years to determine if differential human-dense environments of urban/suburban and rural/farmland locations can influence raccoon roundworm density.

Section 2: Materials and Methods

2.1 - Raccoon Latrine Identification

Latrine sites were identified and sampled across the southeastern region of New-Brunswick in May and June 2021. These sites varied in parameters such as population density (rural – suburban - farmland), presence of food, shelter, as well as domestic wildlife in the form of dogs and cats. Consideration of site locations began through community-engaged science; latrine sites and camera sites were placed following outreach to citizens within the Sackville municipality of New Brunswick through social media platforms, such as community Facebook boards. Raccoon latrines were identified through analysis of fecal characteristics such as length, colour, and presence of >1 scat (Kazacos, 2016). Fecal subsamples were collected in triplicates from each raccoon latrine and subsequently homogenized to create a representative sample of each site. Subsamples were extracted from across the latrine location, with multiple fecal segments within general proximity being included within a single fecal subsample.

2.2 - Wildlife Population Estimation

Raccoon population estimates, in combination with other wildlife factors such as the presence and interaction of other wildlife were determined using night-vision cameras (Reconyx, Hyperfire 2 Trail Camera) installed on private properties in both the Sackville township, as well as nearby rural and farmland regions. Cameras were installed in locations with latrines, as well as those with no-known latrine. Night-vision camera traps serve as a means of mitigating excess costs, energy, and environmental disturbance in suburban regions while not being limited by variable weather conditions (Henschel & Ray, 2003; Rowcliffe *et al.*, 2008).

Locations of installation were selected based on community involvement, messages asking for people willing to allow camera placement was solicited via online message boards (see appendix). This is the most logistically effective way of obtaining permission for many camera placements, but one which creates an initial bias based on the interest of individual property owners/residents. Interest in this project may have been stimulated by notice of prior raccoon activity.

On site, four major factors were considered upon placement of night-vision cameras around a property; 1) Potential access to food sources for raccoons 2) Potential access to shelter for wildlife 3) Presence of competition 4) The property's proximity to road and vehicular traffic levels. No artificial baits or lures were employed for the duration of the study.

Data obtained from cameras include the number of raccoons, domestic animals (cats & dogs), other wildlife (skunks, foxes, deer). Categorical factors such as the presence of food was qualified based on the presence of accessible berries, bird-feeders, and garbage. The presence of shelter was assessed by noting the presence of accessible barns, sheds, storm-drains and crawl-spaces within the location. Each animal sighting was assessed using the assumption that a sighting more than 1-minutes from a previous sighting represented a new sighting.

2.3 - Raccoon Roundworm Egg Isolation

In the isolation of *B. procyonis* eggs for microscopic identification and quantification, PPE was worn at all times, and handling of biohazardous material was performed within a laboratory fume hood (Labconco, Protector Laboratory Hood).

A weighed 5g fecal sample (Acculab, V-1200), initially stored in freezing conditions, was mixed using the addition of 12mL of water and subsequent homogenization through the breaking of clumped-feces until a homogenized-combination remains. This mixture was then filtered through a 7.8-inch mesh-strainer, with an additional 2-3ml of water used to rinse and strain any additional fecal material. The wet fecal matter was pressed until dry to filter any wet-fecal liquid, potentially containing roundworm eggs. Following an autoclave of the mesh-strainer, dried fecal remnants within were discarded into appropriate biohazardous waste. The strained and pressed fecal-water was transferred to a 15mL centrifuge tube (VWR Centrifuge Tube) for subsequent centrifugation using an international clinical centrifuge (International Equipment Co., model CL) using the Cornell-Wisconsin centrifugal floatation technique, at 264Xg for 3 minutes, in accordance with the procedure outlined in Egwang & Slocombe, (1982) and (Thornton *et al.*, 2020). Following an initial centrifugation, excess supernatant, consisting generally of $\frac{3}{4}$ of the initial sample, was discarded to appropriate biohazardous waste, with care taken to not disturb or lose solid sediment at the bottom of the centrifuge tube. A saturated sucrose solution of Sheather's sugar solution, consisting of 1.27g/mL concentration of table sugar (Lantic, granulated sugar) and water, was added to the centrifuge tube and mixed well using an applicator stick between increment additions

of one-half and three-quarters of the centrifuge tube was filled. The remaining Sheather's solution was then added via a medicine dropper to produce a reverse-meniscus. A 22mm square coverslip was carefully placed onto the tube and centrifuged for 5 minutes at 264Xg using the international clinical centrifuge. After centrifugation, the coverslip was removed by lifting straight upwards and placed on a glass microscope slide for visualization, the remaining sugar solution and fecal sample within the centrifuge tube is discarded in appropriate biohazardous waste.

2.4 - *B. procyonis* Egg Identification

Microscopy (Zeiss Axioskop) was performed using a 40X objective and 10X ocular lens for roundworm egg identification, with the 100X objective used for measurements (Zajac & Conboy, 2012). Qualifications for egg identification were based on size of egg, measuring roughly 75µm by 60µm, brown-colouration, as well as the presence of a thick, rough granular shell (Graeff-Teixeira *et al.*, 2016). Care was taken for the potential of egg misidentification due to malformed roundworm eggs, such as any form of eggshell distortions including conjoined eggs, as well as irregular-, crescent-, and triangular-shaped eggs. (Sapp *et al.*, 2018). Following roundworm egg quantification, the number of eggs per gram (EPG) of feces was calculated, using a fecal sample weight of 5g (± 0.5). As outlined by Egwang & Slocombe (1982), fecal-floatation using the Cornell-Wisconsin method has been determined to produce an underestimate of roundworm egg prevalence within fecal samples; as a means of compensating for this underestimate, a standardization factor of 1.6 was applied to the final EPG count (Egwang & Slocombe, 1982). The lower detection limit of this Cornell-Wilson method is 1egg/5g of feces (Egwang & Slocombe, 1982).

2.5 Roundworm DNA-Extraction

DNA-analysis was performed on centrifuged samples by transferring supernatant materials from the coverslip following the centrifugation protocol outlined in 2.3, directly into a 1.5 mL Eppendorf tube. The subsequent DNA-extraction of fecal samples were performed using the QIAamp Fast DNA Stool Mini Kit (Qiagen) protocol following the manufacturer's recommendation for pathogen detection and following raccoon roundworm-specific modifications outlined by Dangoudoubiyam *et al.* (2009), as described below. Supernatant material from coverslip within the 1.5mL Eppendorf tube were transferred to a new 1.5 mL Eppendorf tube

containing a 1mL solution of sterile Phosphate Buffered Saline (PBS) solution to remove the sucrose floatation solution. Following a brief vortexing (Scientific Industries, Vortex-Genie) to disturb the fecal material with PBS, the solution was pelleted by centrifugation at 13,800g for 5 minutes (Labnet, Spectrafuge 24D), with supernatant discarded into biohazardous waste. Following a second wash with PBS and subsequent centrifugation using the same specifications as previously described, the samples were then resuspended in a 180 μ L volume of PBS for further isolation of genomic DNA (Dangoudoubiyam *et al.*, 2009). The sample in 180 μ L of PBS was disrupted with 0.1mm-diameter glass-beads (BioSpec, Cat. No.11079101) and vortexed using a Vortex-Genie (Scientific Industries) for 10 minutes. Following vortexing and care taken to not transfer beads, the vortexed sample was then transferred to a new 1.5 mL Eppendorf tube containing 20 μ L of 20mg/ml Proteinase K, and was incubated overnight in a hot-bath at 55°C for digestion of proteins to take place (Dangoudoubiyam *et al.*, 2009). Following the removal of the tubes from the hot-bath, Buffer AL was added, and the lysate was vortexed and incubated in a hot-water bath at 70°C for 10 minutes to promote cell lysis of potential roundworm cells. A 200 μ L solution of ethanol (95%) was added to the lysate and, following a brief vortexing for 10 seconds (Scientific Industries, Vortex-Genie), the lysate was transferred to a QIAamp spin column placed into a 2mL collection tube and centrifuged at 16,300g for 2 minutes, following the initial centrifugation, the spin-column was transferred to a new 2 mL collection tube. Buffer AW1 and AW2 were individually added and centrifuged at 16,300g for 2 and 4 minutes, respectively, each time replacing the collection tube. The QIAamp spin column was then transferred to a new collection tube and centrifuged at 16,300g for 4 minutes, and finally placed into a 1.5 mL Eppendorf tube with 100 μ L of Buffer ATE added directly onto the QIAamp membrane. Following a 1-minute incubation at room temperature, the tube and spin-column were centrifuged at 16,300g for 2 minutes to elute DNA.

2.6 - PCR amplification of *B. procyonis* DNA

The PCR protocol conducted was unique to each primer set, with respective thermocycler protocols (Table 2.1)

CoI: A 25µL PCR solution was produced through the addition of 2µL of genomic DNA (gDNA) and a 23µL master-mix solution consisting of 8.5µL of nfH_2O , 12.5µL of GoTaq Green Master Mix (GTG) (Promega, #M712C), as well as 1µL of the 10µM *CoI* forward and reverse primer.

Cox-2: A 25µL PCR solution was produced through the addition of 2µL of gDNA and a 23µL master-mix solution consisting of 5.5µL of nfH_2O , 12.5µL of GTG (Promega, #M712C), as well as 2.5µL of the 10µM *Cox-2* forward and reverse primer solution.

Cyt-B: A 25µL PCR solution was produced through the addition of 2µL of genomic gDNA and a 23µL master-mix solution consisting of 5.5µL of nfH_2O , 12.5µL of GTG (Promega, #M712C), as well as 2.5µL of the 50µM *Cyt-B* forward and reverse primer solution.

Following the completion of amplification, PCR samples were electrophoresed on 1.2% agarose gels, with 1X sodium borate (SB) buffer and the addition of 10µL Eco-Stain (Biotech), at 230V for approximately 15 minutes. After completion of electrophoresis, the agarose gel is placed onto a UV transilluminator (Labnet DyNA Light Dual Intensity UV Transilluminator), for visual analysis of DNA bands. Following analysis, agarose gels are discarded into standard waste containment. Amplified DNA were sent to Genome_Quebec for sequencing, these results were subsequently analyzed using FinchTV software to confirm amplification of the *cox-2* primer set.

Table 2.1. PCR protocol for each gene locus. Abbreviated names are as follows: *CoI*, Cytochrome oxidase c subunit 1; *Cox-2*, Cytochrome oxidase c subunit 2; *Cyt-B*, cytochrome b. Protocols were sourced from: *CoI* - (Folmer *et al.*, 1994; Zuccon *et al.*, 2012), *Cox-2* - (Dangoudoubiyam *et al.*, 2009); *Cyt-B* - (Xie *et al.*, 2011).

Target gene	Primer	Sequence (5' → 3')	Amplicon length (bp)	Annealing Temperature (°C)
<i>CoI</i>	CoI F	GGTCAACAAATCATAAAGATATTGG	710	45
	CoI R	TAAACTTCAGGGTGACCAAAAAATCA		
<i>Cox-2</i>	Cox2 F	TGAGTTT TAGTATTCCTGGA	146	48
	Cox2 R	CAGAAGTAATACAAAACCGGAT		
<i>Cyt-B</i>	CytB F	TCC TTA GTA ATG AGT ATT GCG T	548	48
	CytB R	TAT AAC GAC ATT TGA AAA ACA CC		

2.7 - Data Analysis

A negative binomial distribution was employed to produce a regression model mapping *B. procyonis* egg prevalence within latrines based on camera sightings of other forms of wildlife (deer, skunk, fox) and of domestic animals (dog & cat) at each site. Due to the presence of count-data and discrepancies between the mean and variance causing an overdispersion error in the application of a Poisson distribution, a negative binomial distribution was applied (Ismail & Jemain, 2007). Cook's distance was used to determine any data-points skewing the model. Finding data-points being of particular risk, I excluded them following a review of their camera inputs, finding their placement not being uniform to the remaining camera-traps established. These two points were both also of the same latrine location and as such represented a potential pseudoreplication. Upon remaking the model without the risk of pseudoreplication, the overall quality of the model greatly improved, leading to the running the variable inflation factor (VIF) function, in order to determine any risk of multicollinearity. To mitigate the risk of multicollinearity, continuous and categorical factors in the form of raccoon sightings, traffic level, and food presence were omitted. The resultant model used for further analysis consisted of factors of wildlife (excluding raccoons) and domestic animal sightings.

Section 3: Results

3.1 Raccoon Latrine Identification & Wildlife Population Estimation

A total of 7 raccoon latrines were identified across the Town of Sackville and associated regions. Positive latrines were visually identified across 6 latrine locations, with triplicate fecal samples from latrines with lower roundworm EPG count ranging from 0 – 8.3, while latrines with higher roundworm EPG counts range from 76.2 – 159.6 (overall median = 5.40, IQR = 17.6). Most raccoon latrines were found in rural sites located outside of the town of Sackville.

3.2 Ecological Population Determination

A variety of animal sightings were recorded, quantified, and included within continuous factors in conjunction with categorical factors, as outlined in 2.1. Factors of general camera locations and raccoon latrine camera locations were determined (Table's 3.1, 3.2). 943 triplicate animal sightings were recorded from a total of 28 night-vision camera traps set for two-week periods over a 4-month period, from the months of May–August 2021. Examples of successfully identified animal photos are demonstrated in Fig. 3.2.

3.3 *B. procyonis* Egg Identification, Quantification & Confirmation

The most common method for identification of *B. procyonis* and differentiation from other *Baylisascaris* spp. is done through morphological identification. Microscopically identified *B. procyonis* eggs in comparison with CDC images of the parasitic eggs further supports successful microscopic identification (Fig. 3.3) (CDC - DPDx - *Baylisascariasis*, 2019). This method requires considerable expertise in specimen identification via microscopy. In order to provide additional confirmation of roundworm eggs that were microscopically identified, I performed PCR amplification and subsequent sequencing of isolated roundworm eggs. The PCR programs applied initially consisted of the use of three primer-sets (Fig. 3.1). A primer set to amplify the cytochrome oxidase 1 (*coI*) gene is initially used to confirm the presence of animal DNA within the sample (Kunal & Kumar, 2013). Following confirmation of animal DNA, the presence of general roundworm DNA was tested through the use of primers amplifying the cytochrome oxidase 2 (*cox-*

2) gene. Finally primers to amplify the raccoon-roundworm-specific gene of cytochrome B (*cyt-B*) were used to confirm the microscopic identification and differentiation of other roundworm species' (Xie *et al.*, 2011).

Fecal-floated raccoon latrine samples were microscopically analyzed using protocols outlined in 2.3 & 2.4 to identify roundworm eggs. The presence of *B. procyonis* eggs were positively confirmed through PCR reactions using the *Cox-2* primer set. Initial genetic confirmation using *Cyt-B* primer sets were performed, however despite primer optimization through variable concentrations of DNA, primers, as well as by applying a range of temperatures, agarose gel confirmation remained unsuccessful in producing an amplicon. As a result, the roundworm eggs found in this study via fecal sampling of raccoon latrines, were not unable to be genetically differentiated between raccoon roundworm (*B. procyonis*) and skunk roundworm (*B. columnaris*).

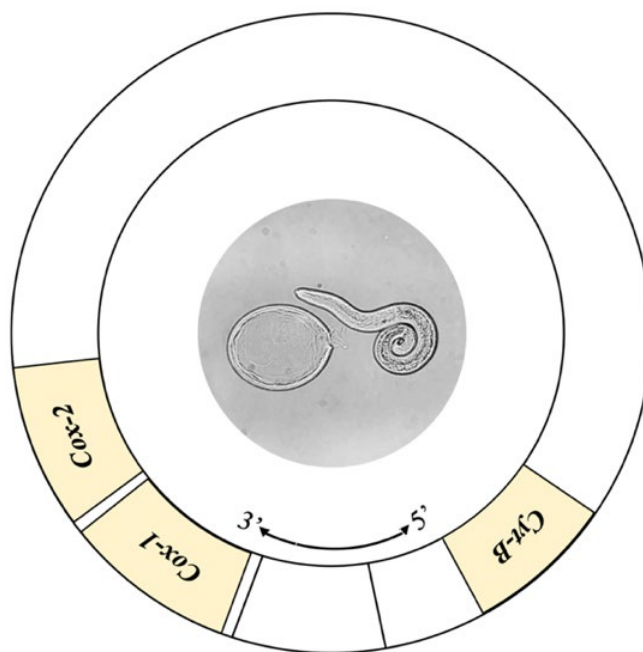


Figure 3.1. Graphical representation of *B. procyonis* mt genome, adapted from Xie *et al.*,(2011). Identified regions include those amplified by PCR reactions. Abbreviated regions are as follows: *Cox-1*, Cytochrome oxidase c subunit 1; *Cox-2*, Cytochrome oxidase c subunit 2; *Cyt-B*, cytochrome b. The direction of transcription is indicated by an arrow (5' to 3').

Successful amplification of the target gene following electrophoresis is demonstrated in Fig. 3.3, which shows DNA PCR products of *B. procyonis*. Agarose gel electrophoresis separates PCR products by size. The predicted size of the *Cox-2* amplicon is ~146 base pairs (Dangoudoubiyam *et al.*, 2009), and is consistent with the results seen (Fig. 3.4). Variation in

amplicons seen in figure 3.4 may be due to resultant variations in amplified DNA, with slight abnormalities in bands correlated to samples with lower query cover, as determined by sequencing (Table 3.3). Raccoon roundworm DNA was considered successfully amplified when a band of approximately this size was visualized from DNA extracted samples collected from raccoon latrines. Genetic sequencing analyses further confirmed successful amplification of *B. procyonis* DNA within three raccoon latrines (Table 3.3). The lower detection-parameter of roundworm amplification with *cox-2* primers is 20-EPG, it was difficult to identify roundworm eggs quantified below this detection limit. Successful microscopic identification of egg-counts greater than the lower detection-limit in tandem with amplification of PCR products enabled quantification of microscopically identified *B. procyonis* eggs in samples below this limit. By analysing the prevalence of *B. procyonis* eggs across latrines sampled, a total of 37.5% of latrines were determined to have roundworm egg prevalence, using the presence of an amplicon as qualifications for prevalence.

3.4 Risk Factor Modeling of *B. procyonis* Prevalence

Figure 3.5 visualizes the negative binomial regression model mapping *B. procyonis* prevalence within raccoon latrines was produced based on sightings of wildlife and domestic animals at latrine locations, as mentioned in 2.7. The model's regression being based on a negative binomial distribution, prevents further validation of the datum's quality of fit within model using an R^2 value. As such, determination of this model's quality was generated through the creation of a set of fitted values as determined by their fitting within the model's parameters, based on the known roundworm egg prevalence (see appendix, Table 3.4) (Fig. 3.5A) (Frisbie, 1980). Upon review of successful model creation, the model was then used to create predicted, or anticipated datapoints of *B. procyonis* egg prevalence at camera locations without corresponding raccoon latrine data, as a means of determining potential regions of high-risk if a latrine were to develop at these locations (Fig. 3.5B). Figure 3.6 shows the geographic distribution of raccoon latrines, their respective roundworm egg prevalence, as well as the predicted roundworm egg prevalence at camera trap locations with no corresponding raccoon latrine data.

Table 3.1 Recorded parameters for each camera locations.*

Camera Sighting Parameter	% of Locations with Sightings
Domestic Animals	52%

Wildlife	39%
Raccoons	78%

*Domestic animal sightings include any appearance of dogs or cats. Wildlife sightings are limited to any animals not qualified under domestic animals or raccoons. Sightings were observed from night-vision camera traps (Reconyx, Hyperfire 2 Trail Camera).

Table 3.2 Parameters associated with identified raccoon latrine locations.*

Location Parameter	% of Locations with Sightings
Domestic Animals	50%
Wildlife	67%
Raccoons	83%
High Traffic	50%
Food availability	100%

*Domestic animal sightings include any appearance of dogs or cats. Wildlife sightings are limited to any animals not qualified under domestic animals or raccoons. Sightings were observed from night-vision camera traps (Reconyx, Hyperfire 2 Trail Camera). Factors of traffic and food availability are categorical; depicted as present/not-present.

Table 3.3 Genetic sequencing analysis of agarose gel (Fig. 3.2).

Latrine fecal sample	Primer	% Query Cover	% Identification	Blast Search
Latrine 3 Sample 1	Forward	43%	100%	MH469665.1
	Reverse	19%	100%	MH469664.1
Latrine 3 Sample 2	Forward	14%	96.3%	MH469665.1
	Reverse	15%	100%	MH469664.1
Latrine 3 Sample 3	Forward	22%	100%	MH469665.1
	Reverse	21%	100%	MH469664.1
Latrine 6 Sample 1	Forward	97%	99.0%	MH469665.1
	Reverse	98%	94%	MH469664.1
Latrine 6 Sample 2	Forward	98%	98.9%	MH469665.1
	Reverse	98%	97%	MH469664.1
Latrine 8 Sample 1	Forward	NA	NA	NA
	Reverse	47%	85.3%	MH469664.1
Latrine 8 Sample 2	Forward	25%	100%	MH469665.1
	Reverse	33%	95.7%	MH469664.1



Figure 3.2 Example photos of animal's captured from night-vision camera traps. Photos are captured at different locations, Reconyx Hyperfire 2 Trail Camera. A) Raccoon sighting B) Deer sightings C) Fox sighting D) Dog sighting

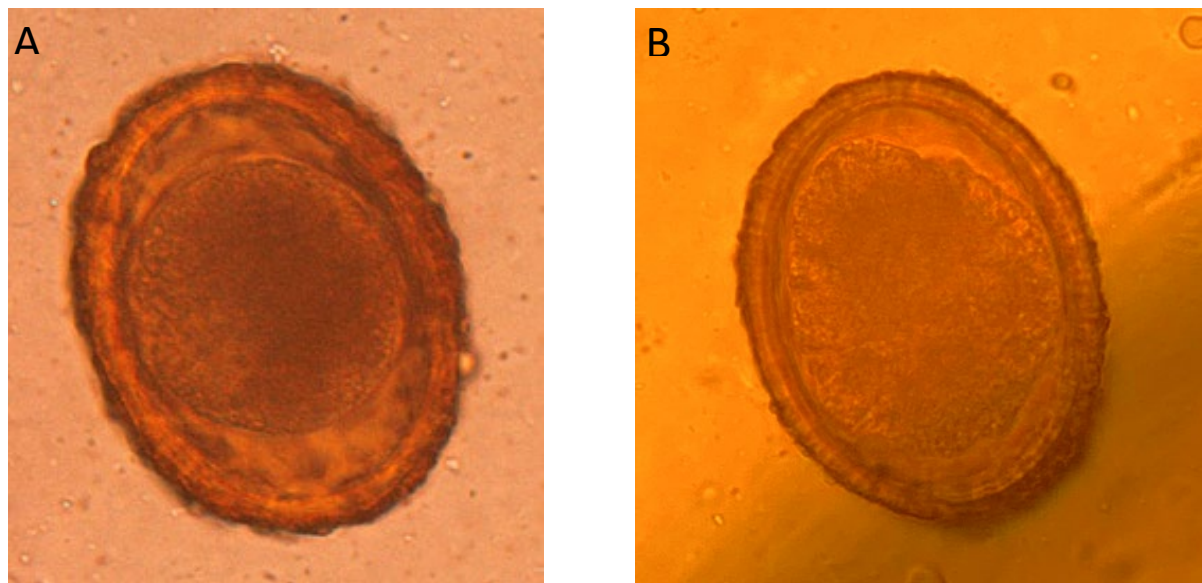


Figure 3.3 Microscopically-identified *B. procyonis* eggs. A) Image taken from CDC website, courtesy of Dr. Cheryl Davis, Western Kentucky University:

<https://www.cdc.gov/dpdx/baylisascariasis/index.html>.

B) Image captured from raccoon latrines sampled with study, photo taken at 400X magnification.

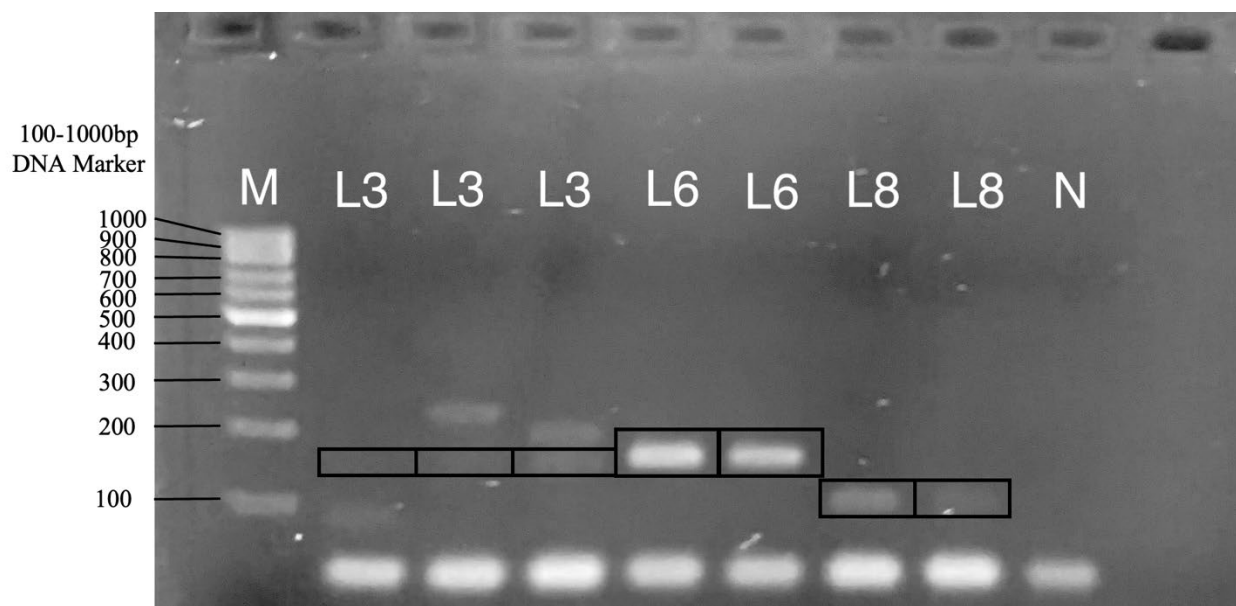


Figure 3.4 Agarose electrophoresis gel of *cox-2* PCR products of amplification of raccoon latrine fecal samples. M lane represent 100-1000bp DNA Marker, L3 lanes represent fecal samples at raccoon latrine 3, L6 lanes represent fecal samples at raccoon latrine 6, L8 lanes represent fecal samples at raccoon latrine 8. N lane represent negative control, examined to ensure lack of contamination. Ladder band sizes are 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100. Amplicons consistent with *B. procyonis* are highlighted. Variability in amplicon size may be due to degraded forms of DNA.

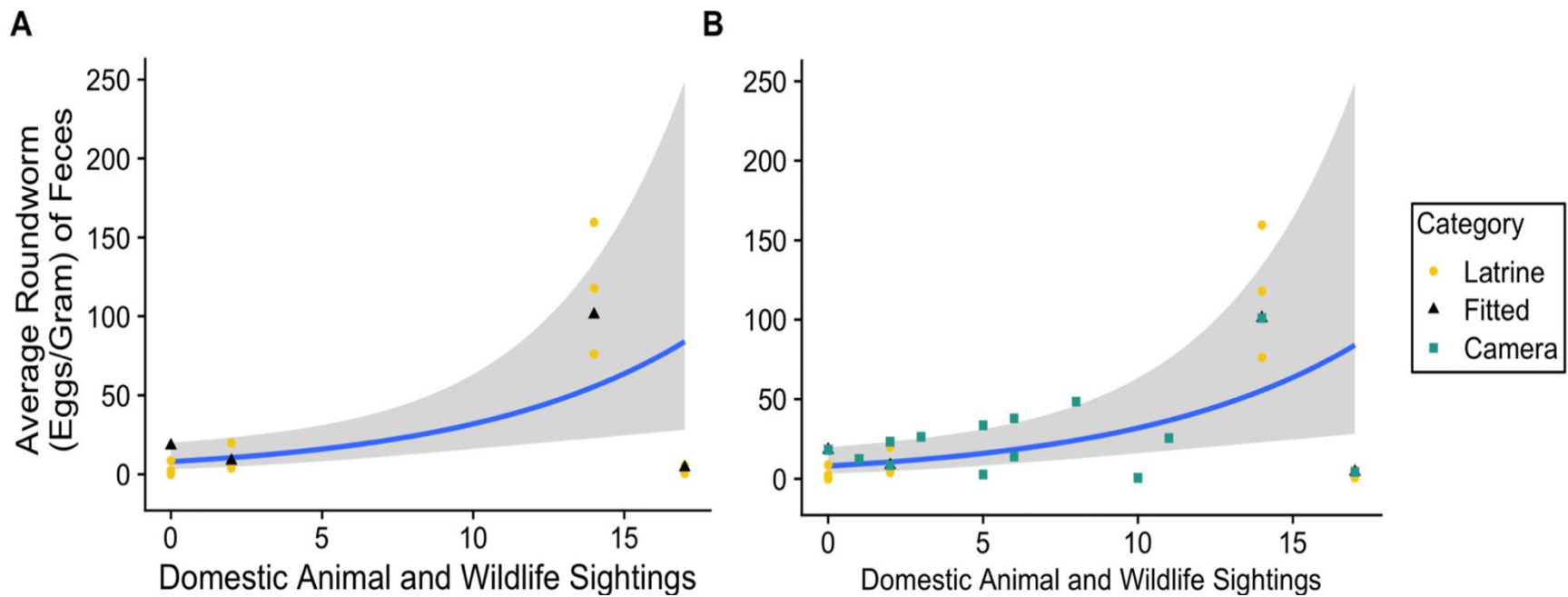


Figure 3.5 Negative binomial regression model depicting A) Quality of negative binomial model; based on fitted *B. procyonis* roundworm egg counts (depicted by black & triangular data-points) extracted from camera-data of actual latrine-sampled locations, and actual *B. procyonis* roundworm egg counts (yellow-data points), being within 95% standard-error bars, as shown by grey-colouration. B) Fit of predicted *B. procyonis* roundworm egg counts at locations with night-vision camera traps (cyan and square data-points) and actual *B. procyonis* roundworm egg counts (yellow data-points). Domestic and wildlife sightings (excluding raccoons) were incorporated to produce the negative binomial regression line seen above in blue. Model visualization was produced using GGPlot2 package on R-studio software.

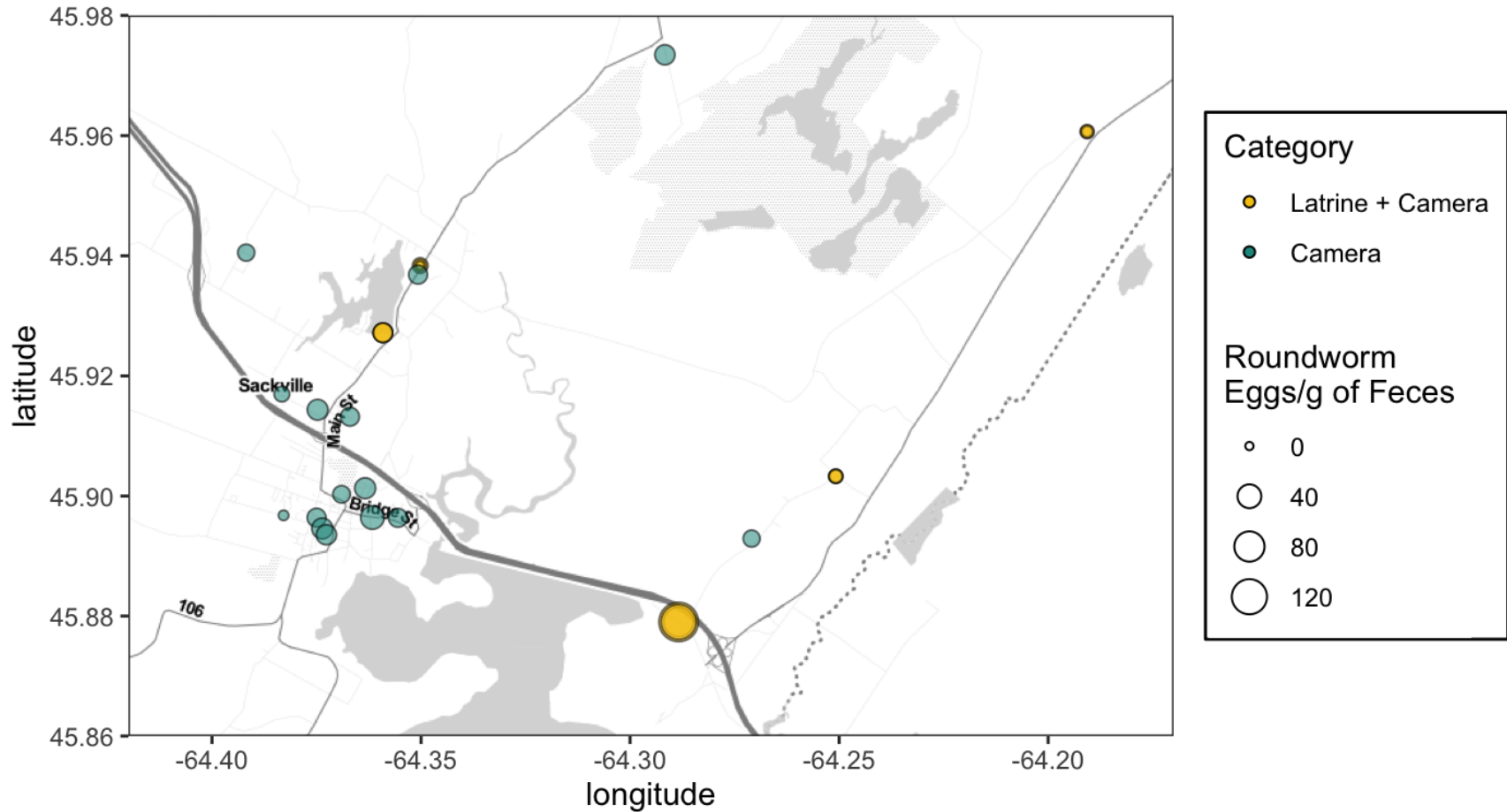


Figure 3.6 Geo-spatial bubble map of *B. procyonis* egg prevalence within region of south-eastern New-Brunswick. Size of data points represent roundworm EPG of feces at predicted/actual raccoon latrine sites. Cyan-coloured “Camera” data-points represent predicted roundworm EPG of feces based on regression model in figure 3.5, using domestic animal and other wildlife (excluding raccoon) sighting data. Yellow-coloured “Latrine” data-points represent actual roundworm EPG of feces at locations with both raccoon latrines and animal sighting data. Model visualization was produced using GGPlot2 package on R-studio software

Section 4: Discussion

4.1 Identification of *B. procyonis* in New Brunswick, Canada

This study further confirms the prevalence of *B. procyonis* in New-Brunswick, Canada and highlights the importance of continued research on a subject following its initial identification. Although this has been previously reported in brief in Kazacos (2016), it remains unpublished in the peer-reviewed literature. Additionally, to our knowledge, this is the first-time quantification of roundworm eggs via non-invasive raccoon latrine fecal sampling was used to determine the parasite's prevalence in the province. Previous analyses, summarized in Kazacos (2016) did so using autopsy samples from raccoons. As such, exact comparisons of previous research of the parasite in New-Brunswick cannot be performed – however, previous studies found a range of 1-97 parasites within host animals in New Brunswick (Kazacos, 2016). Parasitic roundworm egg counts following fecal analysis in this study support these conclusions, further positing a range of 1-159.6 EPG across 8 raccoon latrines sampled. Analysis of latrine sites in southern Quebec generated a range of 1-26,892 eggs per two grams of feces (1-13,446 EPG) (Lafaille, 2011). Prevalence of *B. procyonis* in comparison to Prince Edward Island and Nova Scotia is relatively lower to those determined in this study, suggesting a range of 1-46 parasites within raccoons analyzed by autopsies, examined in previous studies of Nova Scotia (Kazacos, 2016). This study found 37.5% of raccoon latrines were determined to have a prevalence of *B. procyonis* eggs, as confirmed by PCR, while 17 of 24 fecal samples were microscopically identified to be infected with *B. procyonis* eggs. The prevalence found in this study in the 8 sites sampled here is lower than in Quebec, but because of the limited geographic distribution of sites sampled in New Brunswick, it is hard to make broader statements about the relative prevalence. The determination of *B. procyonis* prevalence in this study was performed using the non-invasive technique of latrine sampling. This technique has been found to be proportional to techniques relying upon raccoon dissection (Smyser *et al.*, 2010). This study further confirms the validity of fecal sampling of raccoon latrines as an increasingly effective means of determining *B. procyonis* prevalence, while also enabling further analysis of high-risk areas.

Potential limitations with regards to this study's identification of *B. procyonis* come in the form of both microscopy and PCR analyses. With the lower limit of *cox-2* PCR amplification

being ~20 EPG; samples with a reduced, but still present roundworm egg count (< 20 EPG) following microscopy are unable to appear in genetic analyses (Dangoudoubiyam *et al.*, 2009). While 17 of 24 raccoon latrine fecal subsamples were microscopically analyzed to contain roundworm eggs, only 3 of 8 raccoon latrines were confirmed by positive amplicons (Fig. 3.2). Furthermore, while the cox-2 primer set was able to amplify roundworm DNA, this set is limited in differentiating between *B. procyonis* and *B. columnaris*. Given that fecal samples were extracted from known raccoon latrines, a lack of skunk sightings at positive latrines, and microscopic visualization and comparison to known *B. procyonis* eggs, *B. procyonis* prevalence has been deduced from these results (Fig 3.4).

4.2 Effect of Ecological & Environmental factors on *B. procyonis* egg prevalence

Nematode parasites such as *B. procyonis* that exhibit predator-borne transmission often use their definitive host animals as a means of increasing parasitic mobility and potential egg range (Ewald, 1995). Any negative health effects induced by the parasite causes reductions to host range and so it is hypothesized that to reduce parasitic fitness; less accessible resources for the definitive host will result in less available resources to the parasite (Ewald, 1995; Seppälä *et al.*, 2008). This study found that ecological factors in the form of wildlife (excluding raccoons) and domestic animal sightings were determined as factors influencing *B. procyonis* prevalence. Further analysis of these factors indicates a positive correlation between domestic animal sightings and roundworm egg prevalence, while sightings of other forms of wildlife possess a negative correlation to roundworm egg prevalence. I speculate that these correlations relate to the nature of the interaction between other wildlife versus domestic animals, in relation to raccoon-hosts.

Studies analyzing the limiting factors for raccoons across environmental demographics found that in more rural regions, wherein wildlife is more common, raccoons are limited by resource acquisition, in contrast to urban regions where they are limited by disease prevalence (Prange *et al.*, 2003). Further analyses with respect to predicted and actual *B. procyonis* egg distribution across camera trap locations and latrines sampled in this study, as seen in figure 3.5, show a correlation with the number of domestic animals in more urban locations. In contrast, rural locations possessed greater sightings of other wildlife species. These findings indicate that environments enabling high populations of raccoons and domestic animals are likely indicative of a higher quality of life on parasitic organisms inhabiting a host animal. This increased quality of

life is possibly due to the greater food and resource availability common of urban regions. Rural locations with a high degree of competitors such as skunks and foxes, likely reduce raccoon food-availability, in addition to diluting the potential for parasitic spread (Civitello *et al.*, 2015), possibly resulting in a reduced roundworm egg prevalence.

The greater variability in roundworm egg recovery per gram in more rural locations might relate to the greater geographic range of raccoons in these locations, relative to their densely populated, urban counterparts. Raccoons in rural regions occupy a greater range so have a reduced rate of interaction (Prange *et al.*, 2003). Thus, infected rural raccoons are less likely to encounter others, potentially leading to the greater variability of *B. procyonis* egg prevalence observed across demographic regions.

These findings are supportive of the greater risk of Baylisascariasis contraction with respect to increasing urbanization, thereby increasing overall raccoon populations in close contact with humans. In addition, the reduction of overall biodiversity as a result of climate-change as well as habitat-destruction is likely to reduce the effect of increased biodiversity diluting pathogen spread, thereby increasing the potential for increased pathogen spread (Civitello *et al.*, 2015).

4.3 Future Directions

Further surveillance of *B. procyonis* in New Brunswick is vital to a greater understanding of the potential risk to members of the public. This is particularly important as the anti-helminthic medication used to treat Baylisascariasis, Albendazole, is not currently available in Canada. Future surveillance efforts should be directed at increasing the sample size of raccoon latrines and increasing the range of sampling across the province of New Brunswick and other Maritime provinces. This would allow for validation or modification of the model presented here to provide a predictive tool to detect high-risk locations.

In addition to increasing the overall sample size, the designing of a new primer-set to genetically differentiate past *cox-2* is another means by which this study could further improve. Furthermore, recent serological studies in California have determined the potential for subclinical infections of Baylisascariasis in humans (S. Sapp *et al.*, 2016; Weinstein *et al.*, 2017). Performing similar studies in the province of New Brunswick would be incredibly valuable for further ascertaining the epidemiological risk of contraction within the province.

4.4 Conclusion

This study sought to ascertain the population characteristics of raccoons, domestic animals, and other forms of wildlife within both suburban and rural regions of the Maritimes as a means of comparing the prevalence of the raccoon roundworm (*Baylisascaris procyonis*), assessed through egg counting in latrines. The end goal being to assess the current level of risk on human populations within Maritime Canada. A total of 8 raccoon latrines were identified across the province of New Brunswick, 7 of which sampled from the Town of Sackville and associated regions. Night-vision camera traps were established across 28 locations to determine the population distributions of ecological factors based on animal sightings, in correlation with locations where raccoon latrines were present. Fecal floatation was then performed through centrifugation of fecal samples with a sucrose solution followed by subsequent microscopy and DNA analyses of samples. Raccoon roundworm eggs were microscopically identified across 6 latrine locations, while 3 locations were molecularly confirmed via PCR using the *cox-2* primer set. Triplicate fecal samples from latrines with lower roundworm EPG count ranged from 0 – 8.3, and would be undetectable by PCR analysis, while latrines with higher roundworm EPG counts range from 76.2 – 159.6 (overall median = 5.40 EPG, IQR = 17.6). Most raccoon latrines were found in rural sites located outside of the town of Sackville. Ecological factors of domestic animal sightings and wildlife sightings (excluding raccoons) were successfully correlated, using a negative binomial distribution, to respectively increase and decrease roundworm egg prevalence found within raccoon latrines. Further analysis of these factors produced a predictive model depicting anticipated and actual roundworm egg prevalence at locations both with and without latrines detected. Findings from this study validate previously unpublished research on the prevalence of *B. procyonis* within the province of New Brunswick. Further hypotheses from this study indicates the potential for an increased risk of Baylisascariasis with the onset of urbanization and progression of climate change leading to increased raccoon populations in close proximity to humans.

Bibliography

- Barnes, A. N., Davaasuren, A., Baasandagva, U., & Gray, G. C. (2017). A systematic review of zoonotic enteric parasitic diseases among nomadic and pastoral people. *PLoS ONE*, *12*(11), e0188809. <https://doi.org/10.1371/journal.pone.0188809>
- Bidaisee, S., & Macpherson, C. N. L. (2014). Zoonoses and One Health: A Review of the Literature. *Journal of Parasitology Research*, *2014*. <https://doi.org/10.1155/2014/874345>
- Bozek, C. K., Prange, S., & Gehrt, S. D. (2007). The influence of anthropogenic resources on multi-scale habitat selection by raccoons. *Urban Ecosystems*, *10*(4), 413–425. <https://doi.org/10.1007/s11252-007-0033-8>
- Broadfoot, J. D., Rosatte, R. C., & O’Leary, D. T. (2001). Raccoon and Skunk Population Models for Urban Disease Control Planning in Ontario, Canada. *Ecological Applications*, *11*(1), 295–303. <https://doi.org/10.2307/3061074>
- CDC - DPDx—*Baylisascariasis*. (2019, June 13). <https://www.cdc.gov/dpdx/baylisascariasis/index.html>
- Christou, L. (2011). The global burden of bacterial and viral zoonotic infections. *Clinical Microbiology and Infection*, *17*(3), 326–330. <https://doi.org/10.1111/j.1469-0691.2010.03441.x>
- Civitello, D. J., Cohen, J., Fatima, H., Halstead, N. T., Liriano, J., McMahon, T. A., Ortega, C. N., Sauer, E. L., Sehgal, T., Young, S., & Rohr, J. R. (2015). Biodiversity inhibits parasites: Broad evidence for the dilution effect. *Proceedings of the National Academy of Sciences*, *112*(28), 8667–8671. <https://doi.org/10.1073/pnas.1506279112>
- Cleaveland, S., Laurenson, M. K., & Taylor, L. H. (2001). Diseases of humans and their domestic mammals: Pathogen characteristics, host range and the risk of emergence. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *356*(1411), 991–999. <https://doi.org/10.1098/rstb.2001.0889>

- Dangoudoubiyam, S., & Kazacos, K. R. (2009). Differentiation of Larva Migrans Caused by Baylisascaris procyonis and Toxocara Species by Western Blotting. *Clinical and Vaccine Immunology : CVI*, 16(11), 1563–1568. <https://doi.org/10.1128/CVI.00251-09>
- Dangoudoubiyam, S., Vemulapalli, R., & Kazacos, K. R. (2009). PCR Assays for Detection of Baylisascaris Procyonis Eggs and Larvae. *Journal of Parasitology*, 95(3), 571–577. <https://doi.org/10.1645/GE-1905.1>
- Despommier, D. (2003). Toxocariasis: Clinical Aspects, Epidemiology, Medical Ecology, and Molecular Aspects. *Clinical Microbiology Reviews*, 16(2), 265–272. <https://doi.org/10.1128/CMR.16.2.265-272.2003>
- Dobson, A., & Foufopoulos, J. (2001). Emerging infectious pathogens of wildlife. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 356(1411), 1001–1012. <https://doi.org/10.1098/rstb.2001.0900>
- Egwan, T. G., & Slocombe, J. O. (1982). Evaluation of the Cornell-Wisconsin centrifugal flotation technique for recovering trichostrongylid eggs from bovine feces. *Canadian Journal of Comparative Medicine*, 46(2), 133–137.
- Ewald, P. W. (1995). The Evolution of Virulence: A Unifying Link between Parasitology and Ecology. *The Journal of Parasitology*, 81(5), 659–669. <https://doi.org/10.2307/3283951>
- Ferraguti, M., Puente, J. M. la, Jiménez–Clavero, M. Á., Llorente, F., Roiz, D., Ruiz, S., Soriguer, R., & Figuerola, J. (2021). A field test of the dilution effect hypothesis in four avian multi-host pathogens. *PLOS Pathogens*, 17(6), e1009637. <https://doi.org/10.1371/journal.ppat.1009637>
- Folmer, O., Black, M., Wr, H., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial Cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.

- Fox, A. S., Kazacos, K. R., Gould, N. S., Heydemann, P. T., Thomas, C., & Boyer, K. M. (2010, January 13). *Fatal Eosinophilic Meningoencephalitis and Visceral Larva Migrants Caused by the Raccoon Ascarid Baylisascaris procyonis* (world) [Case-report]. [Http://Dx.Doi.Org/10.1056/NEJM198506203122507](http://dx.doi.org/10.1056/NEJM198506203122507); Massachusetts Medical Society. <https://doi.org/10.1056/NEJM198506203122507>
- French, S. K., Pearl, D. L., Shirose, L., Peregrine, A. S., & Jardine, C. M. (2019). Demographic and Environmental Factors Associated with Baylisascaris procyonis infection of Raccoons (Procyon lotor) in Ontario, Canada. *Journal of Wildlife Diseases*, 56(2), 328–337. <https://doi.org/10.7589/2019-06-153>
- Frisbie, G. A. (1980). Ehrenberg's Negative Binomial Model Applied to Grocery Store Trips. *Journal of Marketing Research*, 17(3), 385–390. <https://doi.org/10.1177/002224378001700314>
- Gavin, P. J., Kazacos, K. R., & Shulman, S. T. (2005). Baylisascariasis. *Clinical Microbiology Reviews*, 18(4), 703–718. <https://doi.org/10.1128/CMR.18.4.703-718.2005>
- Gordon, C., McManus, D., Jones, M., Gray, D., & Gobert, G. (2016). The Increase of Exotic Zoonotic Helminth Infections: The Impact of Urbanization, Climate Change and Globalization. In *Advances in Parasitology* (Vol. 91). <https://doi.org/10.1016/bs.apar.2015.12.002>
- Graeff-Teixeira, C., Morassutti, A. L., & Kazacos, K. R. (2016). Update on Baylisascariasis, a Highly Pathogenic Zoonotic Infection. *Clinical Microbiology Reviews*, 29(2), 375–399. <https://doi.org/10.1128/CMR.00044-15>
- Hamann, K. J., Kephart, G. M., Kazacos, K. R., & Gleich, G. J. (1989). Immunofluorescent Localization of Eosinophil Granule Major Basic Protein in Fatal Human Cases of Baylisascaris procyonis Infection. *The American Journal of Tropical Medicine and Hygiene*, 40(3), 291–297. <https://doi.org/10.4269/ajtmh.1989.40.291>

- Helgen, K. M., Pinto, C. M., Kays, R., Helgen, L. E., Tsuchiya, M. T. N., Quinn, A., Wilson, D. E., & Maldonado, J. E. (2013). Taxonomic revision of the olingos (Bassaricyon), with description of a new species, the Olinguito. *ZooKeys*, 324, 1–83.
<https://doi.org/10.3897/zookeys.324.5827>
- Henschel, P., & Ray, J. (2003). Leopards in African Rainforests—Survey and Monitoring Techniques. *Wildlife Conservation Society*, 216.
- Hof, A. R., Jansson, R., & Nilsson, C. (2012). Future Climate Change Will Favour Non-Specialist Mammals in the (Sub)Arctics. *PLOS ONE*, 7(12), e52574.
<https://doi.org/10.1371/journal.pone.0052574>
- Holand, H., Jensen, H., Tufto, J., Pärn, H., Sæther, B.-E., & Ringsby, T. H. (2015). Endoparasite Infection Has Both Short- and Long-Term Negative Effects on Reproductive Success of Female House Sparrows, as Revealed by Faecal Parasitic Egg Counts. *PLoS ONE*, 10(5), e0125773. <https://doi.org/10.1371/journal.pone.0125773>
- Ismail, N., & Jemain, A. A. (2007). *Handling Overdispersion with Negative Binomial and Generalized Poisson Regression Models*. 56.
- Jacobson, J. E., Kazacos, K. R., & Montague, F. H., JR. (1982). Prevalence of Eggs of Baylisascaris Procyonis (Nematoda-Ascaroidea) in Raccoon Scats From an Urban and Rural Community. *Journal of Wildlife Diseases*, 18(4), 461–464.
<https://doi.org/10.7589/0090-3558-18.4.461>
- Kazacos, K. R. (2001). Baylisascaris procyonis and Related Species. In *Parasitic Diseases of Wild Mammals* (pp. 301–341). John Wiley & Sons, Ltd.
<https://doi.org/10.1002/9780470377000.ch11>
- Kazacos, K. R. (2016). Baylisascaris Larva Migrans. In *Baylisascaris Larva Migrans* (USGS Numbered Series No. 1412; Circular, Vol. 1412, p. 136). U.S. Geological Survey.
<https://doi.org/10.3133/cir1412>

- Kazacos, K. R., & Boyce, W. M. (1989). Baylisascaris larva migrans. *Journal of the American Veterinary Medical Association*, 195(7), 894–903.
- Kazacos, K. R., & Turek, J. (2011). *Scanning Electron Microscopy of the Eggs of Baylisascaris procyonis, B. transfuga, and Parascaris equorum, and Their Comparison with Toxocara canis and Ascaris suuin*. 7.
- Kristen Page, L. (2013). Parasites and the conservation of small populations: The case of Baylisascaris procyonis. *International Journal for Parasitology: Parasites and Wildlife*, 2, 203–210. <https://doi.org/10.1016/j.ijppaw.2013.05.003>
- Kunal, S. P., & Kumar, G. (2013). Cytochrome Oxidase I (COI) sequence conservation and variation patterns in the yellowfin and longtail tunas. *International Journal of Bioinformatics Research and Applications*, 9(3), 301–309. <https://doi.org/10.1504/IJBRA.2013.053613>
- Lafaille, A. (2011). *Le Baylisascaris procyonis dans le sud du Québec: Prévalence chez le raton laveur et étude de contamination des latrines*. <https://papyrus.bib.umontreal.ca/xmlui/handle/1866/6061>
- Lee, A. C. Y., Schantz, P. M., Kazacos, K. R., Montgomery, S. P., & Bowman, D. D. (2010). Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. *Trends in Parasitology*, 26(4), 155–161. <https://doi.org/10.1016/j.pt.2010.01.002>
- Lehmann, T. (1993). Ectoparasites: Direct impact on host fitness. *Parasitology Today*, 9(1), 8–13. [https://doi.org/10.1016/0169-4758\(93\)90153-7](https://doi.org/10.1016/0169-4758(93)90153-7)
- Lutermann, H. (2019). Sex-Biased Parasitism. In J. C. Choe (Ed.), *Encyclopedia of Animal Behavior (Second Edition)* (Vol. 2, pp. 732–738). Academic Press. <https://doi.org/10.1016/B978-0-12-809633-8.90725-8>
- O’Dea, A., Lessios, H. A., Coates, A. G., Eytan, R. I., Restrepo-Moreno, S. A., Cione, A. L., Collins, L. S., de Queiroz, A., Farris, D. W., Norris, R. D., Stallard, R. F., Woodburne,

- M. O., Aguilera, O., Aubry, M.-P., Berggren, W. A., Budd, A. F., Cozzuol, M. A., Coppard, S. E., Duque-Caro, H., ... Jackson, J. B. C. (2016). Formation of the Isthmus of Panama. *Science Advances*, 2(8). <https://doi.org/10.1126/sciadv.1600883>
- Olano, J. P., Weller, P. F., Guerrant, R. L., & Walker, D. H. (2011). Principles of Parasitism. *Tropical Infectious Diseases: Principles, Pathogens and Practice*, 1–7. <https://doi.org/10.1016/B978-0-7020-3935-5.00001-X>
- Page, L. K., Gehrt, S. D., Titcombe, K. K., & Robinson, N. P. (2005). Measuring prevalence of raccoon roundworm (*Baylisascaris procyonis*): A comparison of common techniques. *Wildlife Society Bulletin*, 33(4), 1406–1412. [https://doi.org/10.2193/0091-7648\(2005\)33\[1406:MPORRB\]2.0.CO;2](https://doi.org/10.2193/0091-7648(2005)33[1406:MPORRB]2.0.CO;2)
- Page, L. K., Swihart, R. K., & Kazacos, K. R. (1998). Raccoon Latrine Structure and Its Potential Role in Transmission of *Baylisascaris procyonis* to Vertebrates. *The American Midland Naturalist*, 140(1), 180–185. [https://doi.org/10.1674/0003-0031\(1998\)140\[0180:RLSAIP\]2.0.CO;2](https://doi.org/10.1674/0003-0031(1998)140[0180:RLSAIP]2.0.CO;2)
- Pappaioanou, M., Gonzalez, M. C., Scott, K. A., Tsai, P., & Keusch, G. (2009). Drivers of Zoonotic Diseases. In *Sustaining Global Surveillance and Response to Emerging Zoonotic Diseases*. National Academies Press (US). <https://www.ncbi.nlm.nih.gov/books/NBK215318/>
- Perlman, J. E., Kazacos, K. R., Imperato, G. H., Desai, R. U., Schulman, S. K., Edwards, J., Pontrelli, L. R., Machado, F. S., Tanowitz, H. B., & Saffra, N. A. (2010). *Baylisascaris Procyonis* Neural Larva Migrants in an Infant in New York City. *Journal of Neuroparasitology*, 1(1), 10.4303/jnp/N100502. <https://doi.org/10.4303/jnp/N100502>
- Piero, J., Lorenzo-Morales, J., Martn-Navarro, C., Lpez-Arencibia, A., Reyes-Batlle, M., & Valladares, B. (2012). Zoonosis Caused by *Baylisascaris procyonis*. In J. Lorenzo-Morales (Ed.), *Zoonosis*. InTech. <https://doi.org/10.5772/38883>

- Prange, S., Gehrt, S. D., & Wiggers, E. P. (2003). Demographic Factors Contributing to High Raccoon Densities in Urban Landscapes. *The Journal of Wildlife Management*, 67(2), 324–333. <https://doi.org/10.2307/3802774>
- Prange, S., Gehrt, S. D., & Wiggers, E. P. (2004). Influences of Anthropogenic Resources on Raccoon (*Procyon lotor*) Movements and Spatial Distribution. *Journal of Mammalogy*, 85(3), 483–490. <https://doi.org/10.1644/1383946>
- Preston, D., & Johnson, P. (2010). Ecological Consequences of Parasitism. *Nature Education*, 3, 47.
- Riley, S. P., Hadidian, J., & Manski, D. A. (1998). Population density, survival, and rabies in raccoons in an urban national park. *Canadian Journal of Zoology*, 76(6), 1153–1164. <https://doi.org/10.1139/z98-042>
- Roussere, G. P., Murray, W. J., Raudenbush, C. B., Kutilek, M. J., Levee, D. J., & Kazacos, K. R. (2003). Raccoon Roundworm Eggs near Homes and Risk for Larva Migrans Disease, California Communities. *Emerging Infectious Diseases*, 9(12), 1516–1522. <https://doi.org/10.3201/eid0912.030039>
- Rowcliffe, J. M., Field, J., Turvey, S. T., & Carbone, C. (2008). Estimating animal density using camera traps without the need for individual recognition. *Journal of Applied Ecology*, 45(4), 1228–1236. <https://doi.org/10.1111/j.1365-2664.2008.01473.x>
- Salgado, I. (2018). Is the raccoon (*Procyon lotor*) out of control in Europe? *Biodiversity and Conservation*, 27(9), 2243–2256. <https://doi.org/10.1007/s10531-018-1535-9>
- Sapp, S., Anderson, L., Wilkins, P., Handali, S., Gray, E., Eberhard, M., Woodhall, D., Montgomery, S., Bailey, K., Lankau, E., & Yabsley, M. (2016). Baylisascaris procyonis Roundworm Seroprevalence among Wildlife Rehabilitators, United States and Canada, 2012–2015. *Emerging Infectious Diseases*, 22. <https://doi.org/10.3201/eid2212.160467>

- Sapp, S. G. H., Elsemore, D. A., Hanna, R., & Yabsley, M. J. (2020). Experimental comparison of *Baylisascaris procyonis* definitive host competence between domestic dogs and raccoons (*Procyon lotor*). *Parasitology*, *147*(12), 1344–1351. <https://doi.org/10.1017/S0031182020001122>
- Sapp, S. G. H., Gupta, P., Martin, M. K., Murray, M. H., Niedringhaus, K. D., Pfaff, M. A., & Yabsley, M. J. (2017). Beyond the raccoon roundworm: The natural history of non-raccoon *Baylisascaris* species in the New World. *International Journal for Parasitology. Parasites and Wildlife*, *6*(2), 85–99. <https://doi.org/10.1016/j.ijppaw.2017.04.003>
- Sapp, S., Yabsley, M., & Bradbury, R. (2018). Abnormal Helminth Egg Development, Strange Morphology, and the Identification of Intestinal Helminth Infections. *Emerging Infectious Diseases*, *24*. <https://doi.org/10.3201/eid2408.180560>
- Seppälä, O., Liljeroos, K., Karvonen, A., & Jokela, J. (2008). Host condition as a constraint for parasite reproduction. *Oikos*, *117*(5), 749–753. <https://doi.org/10.1111/j.0030-1299.2008.16396.x>
- Shafir, S. C., Sorvillo, F. J., Sorvillo, T., & Eberhard, M. L. (2011). Viability of *Baylisascaris procyonis* Eggs. *Emerging Infectious Diseases*, *17*(7), 1293–1295. <https://doi.org/10.3201/eid1707.101774>
- Smyser, T. J., Page, L. K., & Rhodes, O. E., Jr. (2010). Optimization of Raccoon Latrine Surveys for Quantifying Exposure to *Baylisascaris procyonis*. *Journal of Wildlife Diseases*, *46*(3), 929–933. <https://doi.org/10.7589/0090-3558-46.3.929>
- Sorvillo, F., Ash, L. R., Berlin, O. G. W., Yatabe, J., Degiorgio, C., & Morse, S. A. (2002). *Baylisascaris procyonis*: An Emerging Helminthic Zoonosis. *Emerging Infectious Diseases*, *8*(4), 355–359. <https://doi.org/10.3201/eid0804.010273>
- Sprent, J. F. (1968). Notes on *Ascaris* and *Toxascaris*, with a definition of *Baylisascaris* gen.nov. *Parasitology*, *58*(1), 185–198. <https://doi.org/10.1017/s0031182000073534>

- Strausbaugh, L. J., Murray, W. J., & Kazacos, K. R. (2004). Raccoon Roundworm Encephalitis. *Clinical Infectious Diseases*, 39(10), 1484–1492. <https://doi.org/10.1086/425364>
- Thornton, G. L., French, S. K., Peregrine, A. S., & Jardine, C. M. (2020). Prevalence of *Baylisascaris procyonis* in raccoon latrines in southern Ontario, Canada. *Veterinary Parasitology: Regional Studies and Reports*, 20, 100392. <https://doi.org/10.1016/j.vprsr.2020.100392>
- Weinstein, S. B., Lake, C. M., Chastain, H. M., Fisk, D., Handali, S., Kahn, P. L., Montgomery, S. P., Wilkins, P. P., Kuris, A. M., & Lafferty, K. D. (2017). Seroprevalence of *Baylisascaris procyonis* Infection among Humans, Santa Barbara County, California, USA, 2014–2016. *Emerging Infectious Diseases*, 23(8), 1397–1399. <https://doi.org/10.3201/eid2308.170222>
- Woolhouse, M. E. J., Dye, C., Taylor, L. H., Latham, S. M., & woolhouse, M. E. J. (2001). Risk factors for human disease emergence. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 356(1411), 983–989. <https://doi.org/10.1098/rstb.2001.0888>
- World Health Organization. (2005). *Combating Emerging Infectious Diseases in the South-East Asia Region*. 46.
- Xie, Y., Zhang, Z., Niu, L., Wang, Q., Wang, C., Lan, J., Deng, J., Fu, Y., Nie, H., Yan, N., Yang, D., Hao, G., Gu, X., Wang, S., Peng, X., & Yang, G. (2011). The Mitochondrial Genome of *Baylisascaris procyonis*. *PLoS ONE*, 6(10). <https://doi.org/10.1371/journal.pone.0027066>
- Yoshida, A., Hombu, A., Wang, Z., & Maruyama, H. (2016). Larva migrans syndrome caused by *Toxocara* and *Ascaris* roundworm infections in Japanese patients. *European Journal of Clinical Microbiology & Infectious Diseases*, 35(9), 1521–1529. <https://doi.org/10.1007/s10096-016-2693-x>

Zajac, A. M., & Conboy, G. A. (2012). *Veterinary Clinical Parasitology* (8th ed.). Wiley-Blackwell.

Zucon, D., Brisset, J., Corbari, L., Puillandre, N., Utge, J., & Samadi, S. (2012). An optimised protocol for barcoding museum collections of decapod crustaceans: A case-study for a 10-40-years-old collection. *Invertebrate Systematics*, 26. <https://doi.org/10.1071/IS12027>

Appendix:

Table 3.4 Roundworm egg prevalence (EPG) distributions for observed, fitted and predicted raccoon latrine samples.

Egg Prevalence	Domestic Animals Sightings	Wildlife Sightings	Category
20	0	2	Latrine
3.7	0	0	Latrine
3	10	7	Latrine
120.9	14	0	Latrine
5	0	2	Latrine
8.5	0	2	Fitted
18.2	0	0	Fitted
4.3	10	7	Fitted
101	14	0	Fitted
8.5	0	2	Fitted
12.46	0	1	Predicted
18.2	0	0	Predicted
18.2	0	0	Predicted
18.2	0	0	Predicted
13.9	4	2	Predicted
4.3	10	7	Predicted
18.2	0	0	Predicted
0.7	1	9	Predicted
26.3	3	0	Predicted
26.3	3	0	Predicted
38.0	6	0	Predicted
18.2	0	0	Predicted
23.3	2	0	Predicted
23.3	2	0	Predicted
33.6	5	0	Predicted
25.6	9	2	Predicted
0.4	0	10	Predicted
18.2	0	0	Predicted
12.5	0	1	Predicted
48.5	8	0	Predicted
101	14	0	Predicted
8.5	0	2	Predicted
2.7	0	5	Predicted

Message board script to community:

“Wanted - People curious about wildlife around their houses at night

My name is Riley Oremush, I am an honours student at Mount Allison University studying human-wildlife contacts and the prevalence of racoons and other animals in rural and urban areas in and around the Sackville area.

I am looking for people willing to put up night-vision cameras around their residences to see if racoons, cats, foxes, coyotes and other critters might be wandering in town at night. The cameras only capture still images and no animals would be harmed. If you are curious about what's in your neighbourhood and, particularly if you have noticed garbage raiding, I'd love to talk to you.

Please contact me at: raoremush@mta.ca”

Link connecting to electronic appendix containing all camera data.