Botfly (*Cuterebra*) Prevalence and Effects in Deer Mice (*Peromyscus sonoriensis*) Populations Within the Kenna Cartwright Nature Park.

Siya S. Markel
Thompson Rivers University

UREAP

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Abstract

This study investigates the prevalence and effects of rodent botfly (*Cuterebra*) parasitism on deer mice (*Peromyscus sonoriensis*) and montane voles (*Microtus montanus*) in the Kenna Cartwright Nature Park, Kamloops, British Columbia. The research focuses on understanding the relationship between *Cuterebra* parasitism and host body condition, sex, and species, leveraging a 24-year dataset (2000-2024) of small mammal trapping conducted by NRSC 4040 students. Data analysis revealed that parasitized deer mice exhibited a statistically higher body mass compared to non-parasitized individuals, while no significant mass difference was found in montane voles. A sex bias was observed in deer mice, with females more frequently found with warbles. The study acknowledges a potential scientific bias due to the seasonal timing of rodent capture, as historical sampling was limited to the fall. This restricted temporal scope may skew the data, potentially missing variations in *Cuterebra* parasitism that could occur at other times of the year. While the limited sample size prevents conclusive findings on the effects of *Cuterebra* parasitism on hosts, the study still provides a crucial baseline by analyzing historical data. This foundation offers valuable insights for future research, particularly in exploring the long-term host-parasite dynamics of this particular ecological context.

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Introduction

Deer mice (*Peromyscus sonoriensis*) and montane voles (*Microtus montanus*) are two rodents in the *Muridae* family that utilize grassland ecosystems as some of their primary habitat (Eder et al. 2011). Deer mice are characterized by their large black eyes, large ears, and bicolored tail, often sporting a rust-colored coat in maturity, while juveniles typically present gray coat colors (Eder et al. 2011). Deer mice are a highly successful species, boasting the title of the most widespread mouse in North America, ranging from coast to coast, as far north as Alaska, and as far south as Mexico (Eder et al. 2011). An omnivorous species, deer mice are able to consume seeds, plant-matter, eggs, insects, and on occasion have been known to eat nestling birds (Eder et al. 2011). Breeding for this species takes place during March and October, and females have multiple young per birth and multiple litters per season (Eder et al. 2011). Conversely, the montane vole, while having a limited range within BC consisting only of the southern interior, stretches as far south as New Mexico (Eder et al. 2011). The Montane Vole is difficult to distinguish from the more widespread Meadow Vole (*Microtus pennsylvanicus*), but is characterized from other *Muridae* by their short ears, blunt snout, and short-legged, stubby appearance (Eder et al. 2011). Highly abundant in its natural range, montane voles provide a relatively reliable food supply for mesopredators (Eder et al. 2011). The portion of the population located in BC represents the montane vole's northernmost range. Their habitat is dependent on the presence of grasses, and the bulk of their diet is made up from grasses, forbs, and sedges. Their mating season is more limited than the deer mouse's, occurring in the spring primarily between the months of March and April, and generally will have one more litter in autumn (Eder et al. 2011). While deer mice are primarily nocturnal, meadow voles can be active at any time of the day and may be influenced by vegetation cover (Watson 2023).

Peromyscus and *Microtus* populations are known for having variable populations, with *Microtus* being known for multi-annual fluctuations which peak every 2-5 years (Sullivan et al. 2021), whereas *Peromyscus* are known to be more annually variable. *Peromyscus* populations seem to be seasonally variable, with die-offs and declines occurring in summer and winter during harsh conditions (Sullivan et al. 2023). Small mammals such as deer mice and meadow voles form the basis for the food systems they exist in, often the main source of food for larger predators such as hawks, owls, snakes, coyotes, and many other mammals (Quinn et al. 2012). Due to their abundant nature (Eder et al. 2011), they provide a consistent source of food for predator species that can be found within the bunchgrass and ponderosa pine biogeoclimatic zones. Many of the mesopredators that utilize these two species are imperiled themselves, such as the Western Rattlesnake (*Crotalus oreganus*), Burrowing Owl (*Athene cunicularia*), and American Badger (*Taxidea taxus jeffersonii*) (ESDC 2023).

Grassland ecosystems, where deer mice, montane voles, and the predators that subsist off them reside, are under pressure from climate change and anthropogenic disturbance, and are decreasing at a rapid rate (White et al. 2011). While grasslands are a historically resilient ecosystem, and are known to be particularly resilient to changes in temperature, exceptions occur during climate events such as extreme heatwaves (White et al. 2011). In a study done by Bai et al. (2022), an examination of four different grassland systems within northern China showed that monthly climate variables had a greater effect on plant diversity than annual averages. They discovered that an increase in temperature in the monthly temperature minimums decreased plant diversity, whereas in other areas an increase in monthly temperature averages had a positive effect on plant diversity within that area. The change in diversity was highly dependent on the plant species within the area, and found that future efforts to categorize and study plant diversity in the wake of climate change based on temperature may be more effective when monitoring on a monthly basis rather than looking at annual averages within an ecosystem. This means that increased temperature variability, or record high and low temperatures within short-term events such as heatwaves and polar vortexes, can have a very dramatic effect within grassland ecosystems, which can be more easily observed and understood with consistent examination on a monthly basis. A study completed by Fartmann et al. (2021) on climate warming driving distribution of grassland grasshoppers found that the study site in Germany had a 1.1°C increase in temperature through the years of 1996-2011. These warming trends are only expected to increase; Kreyling et al. (2019) predicts that winter warming will increase by an additional 2°C in central Europe by 2071-2100. Grassland ecosystems are some of the world's most imperiled systems, and face biodiversity loss through human development, pollution, and both invasive and agronomic species (Peng et al., 2022). However, they have found that climate change continues to be the singlemost driving factor of degradation within these ecosystems. For keystone predators, a loss of habitat or changes in prey composition due to climate change creates new difficulties with conservation. These difficulties increase when the very basis of the food web also becomes imperiled. Deer mice have been shown to have survival rates that are highly correlated with precipitation (Sullivan et al. 2023), showing declines with only a small decrease in mean precipitation. The southern interior of British Columbia has seen not only drought, but several significant heat waves within the last five years (MWLRS [date unknown]). While influences from climate change on deer mice may not yet be clear, it is a point of concern given our knowledge of this species' ecology. A continued trend of drought and decreased mean precipitation could cause declines in a species that has been a stable source of food for imperiled predators, providing another source of pressure that could devastate populations or conservation attempts.

One of these grassland ecosystems has been the subject of a long-term small mammal trapping project since 1997. The NRSC 4040 Wildlife Management and Conservation 1: Theory and Principle class at Thompson Rivers University has been trapping the Kenna Cartwright Nature Park in Kamloops, BC since 1997. Within the Kenna Cartwright Nature Park area, there are three biogeoclimatic zones. The park consists of the PPxh2, BGxh2, and the BGxw1 (iMapBC 2018). The bunchgrass (BG) biogeoclimatic zone is characterized by warm/hot, dry summers, and moderately cold winters. Little precipitation within this zone means that most of the moisture for plant growth comes from winter snowfall (Lloyd et al. 1990). Climax sites within the Kamloops region are dominated by bluebunch wheatgrass (*Pseudoroegneria spicata*), with smaller components of sandberg's bluegrass (*Poa secunda*), rough fescue (*Festuca altaica*), big sage (*Artemisia tridentata*), and sparse or singular poles of ponderosa pine (*Pinus ponderosa*) (Lloyd et al. 1990). The BGxh occurs below 700m, colloquially referred to as "lower grassland", whereas the BGxw occurs above 700m as the "middle grassland" (Lloyd et al. 1990). The ponderosa pine (PP) biogeoclimatic zone occurs in low elevations, and is characterized by dry and hot summer conditions, making it the warmest biogeoclimatic forested zone. Forest composition commonly consists of parkland, savannah-like stands of ponderosa pine dominated by bluebunch wheatgrass in the understory (Lloyd et al. 1990). Smaller components of interior douglas-fir (*Pseudotsuga menziesii var. glauca*) and trembling aspen (*Populus tremuloides*) are present in the overstory on sites with more moisture, with arrow-leaved balsamroot (*Balsamorhiza sagittata*), yarrow (*Achillea millefolium*), and timber milk-vetch (*Astragalus miser*) (Lloyd et al. 1990) in the understory. The PPxh2 is one of the two variants of the PPxh, designated by the presence of fescues, and is the variant that occurs in the Thompson and Nicola regions (Lloyd et al. 1990).

Figure 1. Map showing the usual locations of where the NRSC 4040 trapping grids are set each year. (iMapBC 2018)

Three trapping grids were initially established, with a fourth "overflow" grid being added later on as class sizes grew. These grids are colloquially referred to as "Weighscale", "Dufferin", "Campus", and "Bunker", with the Bunker grid being the aforementioned overflow grid. Small mammals provide an easy, hands-on way of learning wildlife field techniques and data analysis, making the Kenna Cartwright Nature Park grids a perfect laboratory exercise for the NRSC 4040 course. As a result, trapping data that includes sex, species, and weight of the small mammals captured exists in paper-record from roughly the year 2000-2024. Notably, throughout this twenty-four year record, records exist of botfly parasitism on both deer mice and montane voles. Botflies are a group of insects in the family *Oestridae* of order *Diptera* (Rogers 2024). Of the four families in genus *Oestridae*, one, *Cuterebra*, is known for infecting rodents in the Americas (Slansky et al. 2002). Within the genus *Cuterebra*, rodent and lagomorph botflies, three species have been found in BC (ISU n.d.). These species are *Cuterebra approximata, Cuterebra fontinella, and Cuterebra tenebrosa* (Cannings 2022). Laying their eggs near activity areas of potential hosts, *Cuterebra* larvae will hatch in response to body temperature increases and enter the host via wounds or orifices (Timm et al. 2022). It is then that the larvae will move through the body to a suitable site for development, thus creating a “warble”, an open pore created by the insect that is used for breathing and excretions (Slansky et al. 2002). It has also been seen that infection prevalence was greatest near forest edges, given that botflies prefer mating in open areas (Wolf et al. 2001). It was thought by many that infestations of *Cuterebra* could be detrimental to the host species, given the large size of warbles created and the fact that many *Cuterebra* larvae could infect one host at a time (Timm et al. 2022), but many have found that is not the case. Generally, the infestations of *Cuterebra* tend to be benign, in that there are no positive or negative effects to the host species (Timm et al. 2022, Cramer et al. 2006). *Cuterebra* has limited research surrounding it, most studies occurring in the southern USA and few populations consisting of deer mice or voles have been studied. Most studies or data collection seem to have occurred in the 1990s-2000s (Cramer et al. 2006, Timm et al. 2022, Wolf et al. 2001) when climate variables may not have been as dramatic as they are today. Understanding how the effects from different environmental factors, including the parasitic relationship of *Cuterebra* on these species, influence individuals within a population helps to provide a framework that managers can use to best protect these ecosystem webs.

Because no prior studies have been done on the Kenna Cartwright Park population in regards to the abundance or effects of *Cuterebra*, it may be important to begin collecting data on the sub-family. Major declines in insect populations and biodiversity have become common throughout the world, due to agricultural industrialization, pesticide and fertilizer usage, and the promotion of monoculture agricultural and timber crops (Forister et al. 2019). However few analyses have been done on *Oestridae's* reaction to climate change, as to whether or not the increasingly variable climate conditions have been negative or beneficial to the species existing within the area. Because little is known about *Cuterebra's* habitat preferences, apart from the fact that proximity to the host species of choice seems to be the most limiting factor, establishing a baseline in populations utilizing a host could lead to further research later on. This study aims to answer some preliminary questions on *Cuterebra's* presence within the Kenna Cartwright Nature Park, and the NRSC 4040 trapping population. Is one species of rodent being found with more botflies than others? Is age or gender bias occurring with Cuterebra infestation? And are previously infected rodents being recaptured, and at what rate?

Methodology

Trapping was conducted throughout the months of May and June in the Kenna Cartwright nature park alongside the work of Master's student Kara Atkinson. At each trapping location, a four by fifteen station grid was set up, totalling to sixty Longworth style traps at each site. The Bunker grid was trapped in early May from the 17th to the 19th, while the Weighscale, Dufferin, and Campus grids were trapped from July 22nd to 24th. Because trapping occurred alongside Kara Atkinson's project, the month of June was used to analyze historical data, and help trap grids set up in other locations for her Master's thesis. Each trap is set with synthetic bedding and a plastic cover to protect from the elements. Traps are locked open and baited with a mixture of sunflower seeds and rolled oats for three nights, before traps are set to snap shut upon entry for three nights. Traps were set within two hours of dusk, and checked within two hours of dawn the following morning. Rodents were processed upon capture, equipped with a 4-digit ear tag, and had body measurements taken. Body measurements included head width (taken with a caliper), foot length, ear length, body mass (taken with a Triton T3 scale), and temperature. Temperature was taken using a digital, laser thermometer on the abdomen, allowing for a non-invasive way of taking temperature readings out in the field (Kawakami et al. 2018). This method of taking temperature readings was chosen in favor of others due to anecdotal evidence provided by Kara Atkinson; because most observed warbles seemed to be on the buttocks, there was a concern that other methods like a rectal temperature gauge wouldn't work if genitals were obscured by a warble.

Analysis of body condition was done using the Scaled Mass Index (SMI) (Peig et al. 2009) equation, with the intention to compare the parasitized population to the non-parasitized, control population captured this season. However due to limited sample size in the 2024 field season, comparisons could not be accurately made between the treatment and control groups with the Scaled Mass Index equation. Historical data was analyzed instead, which only included the body mass measurements taken in the NRSC 4040 class. Comparisons between the parasitized and non-parasitized population were made using the Welch's two-sample t-test, with separate tests performed for each species. The Welch's two-sample t-test was chosen due to the unequal variances and unequal sample sizes occurring in the treatment and control groups. Differences between warble prevalence based on sex, and locations of warbles on the body, were graphed using a histogram with the ggplot2 package in R Studio. Locations of warbles on the body were divided into categories based on the following diagram (Figure 2, below) by Wingerd 1988.

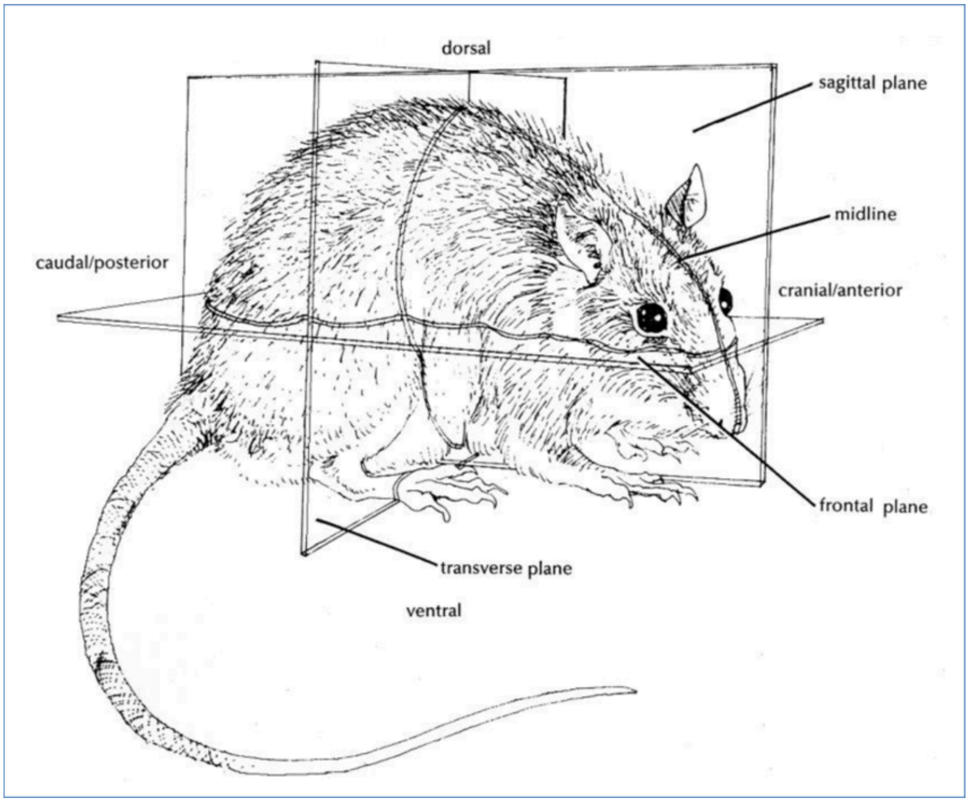

Figure 2. A diagram showing the anatomical planes of a rat. Wingerd 1988.

Cuterebra warbles that occurred on the buttocks, genitals, or hips were lumped into the “Caudal” category. Warbles that occurred on the back or shoulders were considered “Dorsal”. Warbles on the belly or underside of the body were considered “Ventral”, and those occurring on the face considered “Cranial”. Warbles occurring on the legs were counted as their own categories, being “Front Legs” and “Back Legs”, due to the potential inhibitions that having a warble present in that location could present.

Results

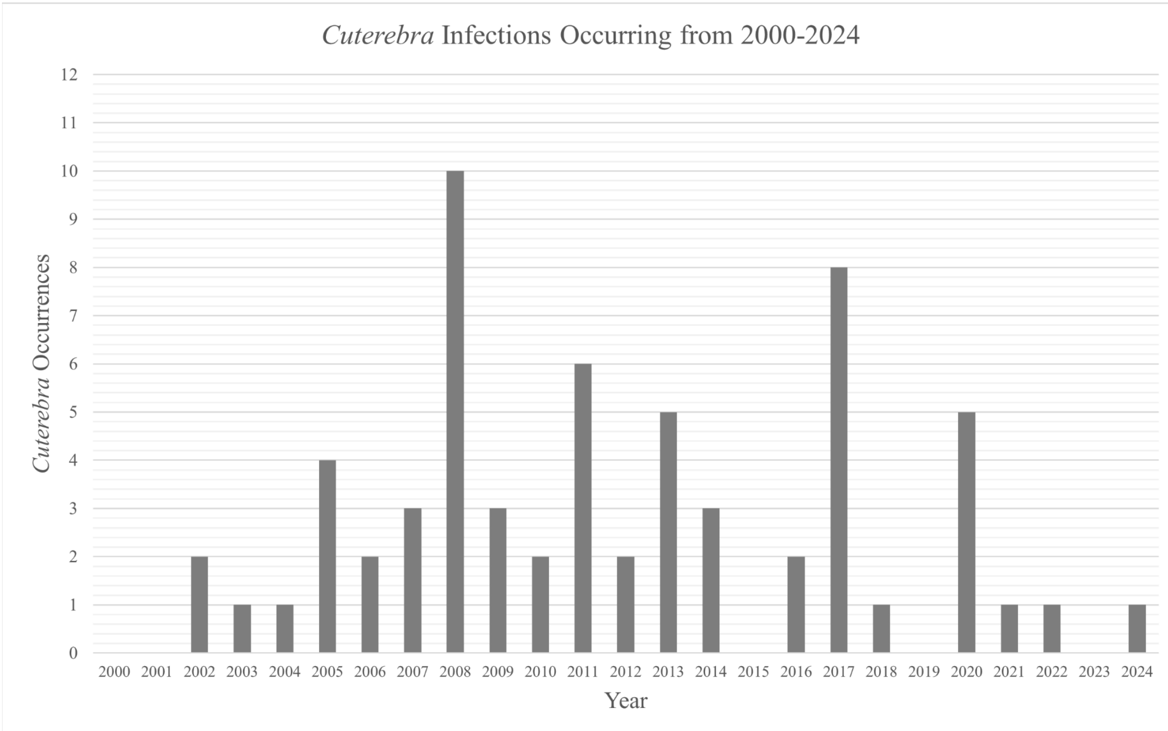

Figure 3. *Cuterebra* parasitism occurring from 2000-2024.

Data from 2000-2024 was used to analyze and make body weight comparisons between the portion of the population parasitized by *Cuterebra* larvae, and those that were not. Most historical trapping occurred at the very end of September, or first week of October, towards the end of the *Cuterebra* active period (iNaturalist 2024). An exception occurred in 2008, when trapping occurred in June and September of that year. The year with the highest number of warbles seen was 2008, with a total of 10 *Cuterebra* warbles observed across both trapping periods. The second highest occurred in 2017, with 8 *Cuterebra* warbles observed across one late September trapping period. No *Cuterebra* warbles were observed in the years of 2000, 2001, 2015, 2019, and 2023. In total, over the course of the twenty-four trapping years, sixty-three warbles were seen on either deer mice or montane voles.

Historical data from 2000-2024 showed that the portion of the population infected with *Cuterebra* in Deer Mice had a statistically greater mass than the population without, falling in line with previous assumptions. The Welch's two-sample t-test performed on the population of Deer Mice resulted in a t-statistic value of -3.9079, 52.749 degrees of freedom, with a p-value of 0.000267. The test performed on the population of Voles resulted in a t-statistic value of -1.2512, 6.0756 degrees of freedom, and a p-value of 0.2569. The critical value calculated for the deer mice using the qt function in R Studio was 2.005969, rejecting the null hypothesis. The portion of deer mice captured with warbles have a statistically greater mass than those without. Montane voles, however, had a critical value calculated using the qt function in R Studio of 2.43955. This confirms the null hypothesis, and means that the portion of deer mice captured with warbles do not have a statistically different mass than those without.

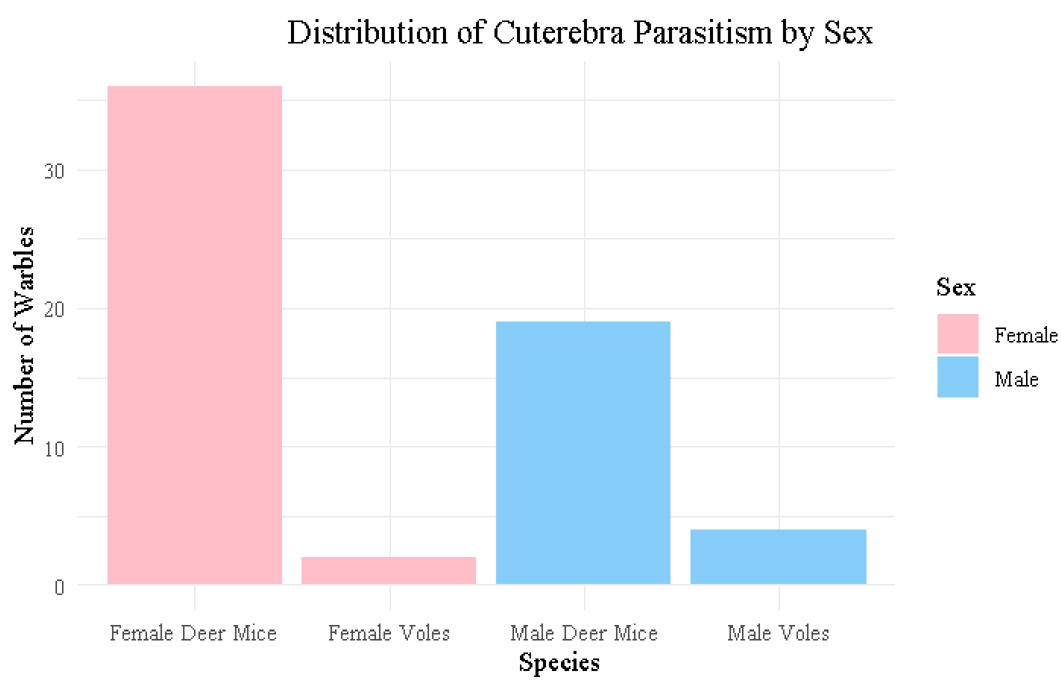

Figure 4. Distribution of warbles present based on sex and species.

A histogram of the percentage of female vs male individuals with warbles in deer mice and montane voles was created using the ggplot2 package in R Studio. In deer mice, 65.4% of

Cuterebra infestations occurred in female mice, while 34.5% occurred in males. Conversely, 66.6% of infestations occurred in males in montane voles, while 33.3% occurred in females. Only one montane vole, due to being dead in the trap, was unable to be sexed. Most of the warbles occurred in deer mice, making up 90.3% of all parasitized individuals. Montane voles only made up 9.68% of all parasitized individuals, only seven individuals having been found with warbles in the entire twenty-four year trapping period.

In the historical records, the rate of recapture could only be studied in the 2008 year, where two trapping periods occurred. Overall there was a 20.5% recapture rate for individuals that were tagged in the summer showing up again in the fall. Of those individuals, only one had warbles in the previous trapping session. The individual captured in the 2024 trapping season on the Weighscale grid was recaptured the day after release without the warble present, having had the larvae exit the pore sometime between the mornings of the 23rd and 24th.

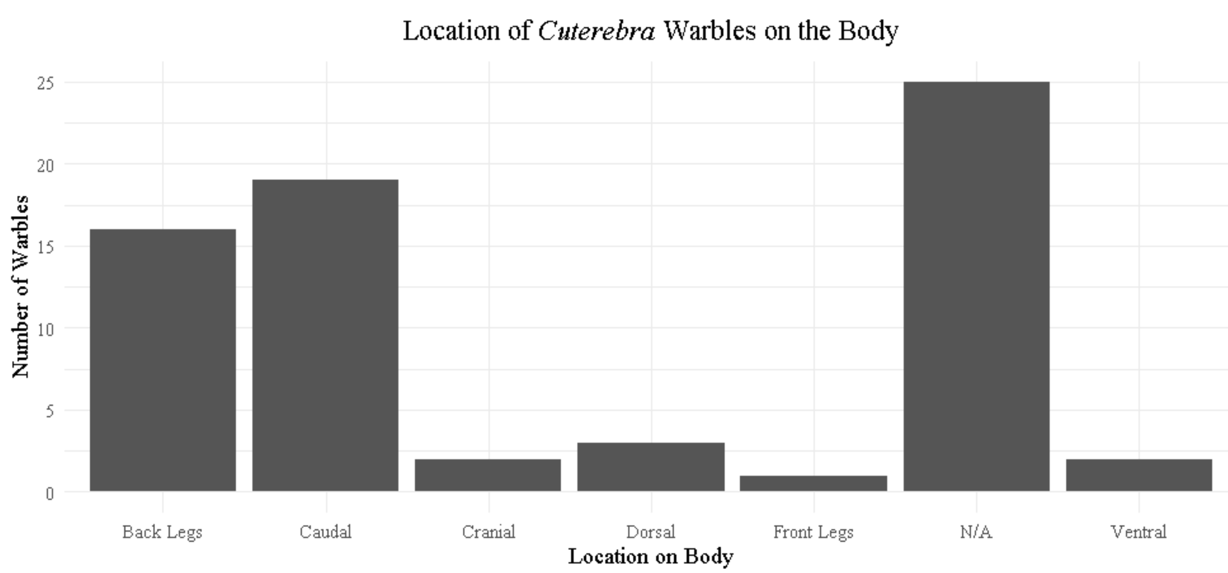

Figure 5. Locations of Warbles on the Body Across Both Species.

While the historical data often lacks the location of the warble on the body, as shown above in category “N/A”, most of the warbles occurred on the posterior end of the body, on the hips, buttocks, or rear legs. The second-most prevalent area was the dorsal plane, on the back or shoulders. The only individual to be captured in the 2024 year had a warble in this location. Of the sixty-two individuals with warbles, four individuals were found with evidence of past warbles in the form of circular scars where the entrance pore would've once been. The only individual found with a warble in 2024 was a female with the tag 4401, who had a warble present on her left side.

Figure 6. Individual 4401 with a *Cuterebra* warble seen on her left side. The flesh-colored protrusion is the warble pore.

Individual 4401 (Figure 6, above) was alert and relatively calm during the handling process, however attempts to remove the warble for sequencing were unsuccessful and ceased to reduce stress to the animal. Individual 4401 was parasitized by multiple species, and was found with a tick on her neck, and a tapeworm on her anus. The tick and segment of the tapeworm present were removed. Individual 4401 was released following processing and attempted removal of the *Cuterebra* larvae, and was recaptured the next day without the larvae present.

While Individual 4401 was reproductively active, showing visible nipples that indicated lactation, palpation of the abdomen to determine whether or not she was pregnant wasn't possible given the location of the warble on her abdomen.

Discussion

The most interesting observations in this study were found in *Peromyscus*, particularly in that warble-infected mice were statistically heavier than those without, and that there was a higher demographic of female parasitism than male. Generally, sex bias for hosts has not been observed in *Peromyscus* (Cramer et al. 2006, Johnson et al. 2018), which makes the findings of this study particularly interesting. Brown and Fuller (2006), for example, found that on floodplain environments, *Cuterebra* warbles were more predominantly found in male white-footed mice (*Peromyscus leucopus*). However, the Kenna Cartwright Nature Park grids all exist at elevation, far above any risk of flooding from the Thompson Rivers. It is unclear why a bias may be occurring towards female deer mice, especially over a long time period. The historical data also rules out the theory that females could simply be more predominant on the grids and thereby reflecting the disparity seen in parasitism; of the 4,248 individuals sampled, 2,177 were female, accounting for 51.2% of the population. While a similar difference occurred in the voles, the sample size is too small to conclusively determine whether or not this is a pattern.

Welch's two-sample t-test saw that deer mice with warbles were statistically heavier than those without. This is an unsurprising result, as third instar *Cuterebra* warbles take up a significant portion of space within the body cavity. A study by Cogley (1991) stated that in deer mice, warble volume grew from 0.05ml to 0.68ml maximum over the course of the study. With some mice on the Kenna Cartwright Nature Park grids having up to two warbles at a time in the historical data, presuming the *Cuterebra* larvae here grow at a similar rate, that could provide at least an additional gram of body weight. For small or young mice, that could be a significant portion of their body mass. Cramer et al. (2006) theorized that an increase in body mass post-infection could've been white-footed mice compensating for the nutrients taken from them by the parasite. They stated that unless the mice switched to a high-protein source of food during infection, they would then have had to compensate by eating more food, thereby increasing their fat stores. While this potentially explains why we see a statistically significant difference between the deer mice, the same correlation is not made with montane voles. While there may be bias given the low numbers of montane voles found with warbles (only seven), this may be more easily explained by the weight differences between the two species. Montane voles weigh quite a bit more than deer mice. The largest parasitized montane vole was 42.5g, the smallest being 19.0g. Comparatively, the smallest deer mouse trapped with a warble was 12.0g, the largest being 32.5g. It is reasonable to assume that the lack of significance between the two groups is partially due to sample size, but also the fact that a warble would make up a significantly smaller portion of a montane vole's body mass than it would a deer mouse.

The locations of warbles on the body are consistent with literature, the majority of known locations occurring in the posterior region of the body. Cogley (1991) encountered similar findings, stating that warbles were almost entirely found in the inguinal region of the body. However it is worth noting that warble location does vary based on the species of *Cuterebra* creating the warble. The species in Cogley (1991) was *C. fontinella*, and while that species is thought to be present here in the Southern Interior, DNA sequencing would be needed to say for sure. While an extraction of the larvae in individual 4401 was attempted for this purpose, the location on the body made it difficult to physically maneuver the larvae out of the pore. Efforts were eventually ceased in order to preserve the health and wellbeing of the host.

The historical highs and lows of *Cuterebra* presence in the host species, as well as the years where they were absent from trapping, can be explained due to the flight periods of botflies. Predominant years, such as the 2008 year, had trapping that occurred in summer during their most abundant periods. Otherwise, all trapping sessions occurred in the last week of September, or first week of October. This part of the year is the very end of their active season, after which larvae will overwinter in the soil to pupate (UoS 2021). This does create bias in the samples, as generally the population is only being surveyed at the very end of their active season. In some years, we can deduce that the lack of *Cuterebra* warbles being found is due to an earlier end of the active season, likely due to colder temperatures. Adult botflies need at least 23℃ for initiation of flight (Cramer et al. 2006), and while Kamloops is known for maintaining warm temperatures throughout the months of September and October, these months can have variable weather and can have earlier onsets of colder temperatures.

Overall, of the 4840 individuals sampled over the 24-year study period, only 63 were found to be infected with *Cuterebra* larvae, consisting of only 1.3% of the population. It is reasonable to say that at a population level, parasitism by *Cuterebra* larvae is likely having little to no effect on population demographics. However, it is highly likely due to the bias given by the historical data towards the end of the active season that we are not seeing the full picture of *Cuterebra* activity. Trapping at other points of the year would likely not only increase the amount of individuals surveyed, boosting estimated population numbers, but also increase the amount of *Cuterebra* parasitism that is being observed.

Conclusion

Cuterebra is a genus that remains understudied within the Kamloops region and British Columbia as a whole. Many aspects of this genus, including its behavior, taxonomy, and effects on hosts, still require dedicated research. This study provides intriguing insights into the parasitism of *Peromyscus*, particularly the unexpected higher prevalence of warbles in female mice and the significant increase in body weight of infected individuals. However, while this research offers a valuable baseline of information, it cannot definitively determine the broader impact of *Cuterebra* on local *Muridae* populations. I recommend that future studies be conducted during the active season of *Cuterebra* and span multiple seasons to account for potential seasonal variability. Additionally, it would be beneficial to include body-length measurements of the hosts at the time of trapping. This would enable the use of a Standard Mass Index in future studies, allowing for more accurate comparisons of body condition across different treatment groups. Among the length measurements tested, head width, measured with a caliper, proved to be the most reliable for correlating with the weight and size of the individuals. In contrast, ear and foot length did not provide accurate reflections of the host's overall size and condition. Generally, the ears and feet were “grown into”, and were quite large even in juveniles. The Kenna Cartwright Nature Park populations present a promising opportunity for future studies, which could explore these dynamics more thoroughly and contribute to a deeper understanding of the host-parasite dynamics in this specific ecological context.

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