Impacts of Geographical Variation and Time Elapsed on Nymph Distribution Across Various Animal Host Species in the Twin Cities Metropolitan Area.

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Background information

The tick lifecycle consists of four phases, egg, larvae, nymph, and adult. During the nymph phase, the tick carries the highest likelihood of transmitting a tick-borne disease to a human being due to various factors[1]. Over a period of 18 years, nymphs were collected from the 7-county metropolitan area in Minnesota by the Metropolitan Mosquito Control District (MMCD) from a variety of host species[2]. On this existing collection of extracted tick DNA, I performed PCR, gel electrophoresis, and statistical analysis in order to observe the proportion of nymphs positive for lyme disease, and evaluate trends in the number of nymphs found on each host species across each county and the effect of time on the number of nymphs found on each host species.

Observing trends in vectors of infectious diseases, along with the diseases themselves, is a common and extremely important component of public health, and this strategy is often employed with ticks. In a study performed in 2022, ticks were collected from domestic animals in Ghana and their DNA was extracted and analyzed for any pathogens present. Statistical analysis, including Pearson chi-square and Fisher's exact test, were utilized to determine any "association between tick species and ecological zone"[3], Pearson chi-square was also used to investigate association of infection status with "animal host and ecological zone"[3]. Furthermore, in study from 2025, ticks were collected from gardens in Belgium, identified, and analyzed for pathogens. Utilizing a generalized linear mixed effects model, the data gathered was then analyzed based on "tick prevalence and pathogen density"[4]. Although these studies contribute important information about the presence of ticks and tick-borne pathogens to their respective geographic regions, less is known about overall trends in host species, and even less about these trends in the Twin Cities.

Methods & Results

In order to begin investigating the association, or lack thereof, between nymphs, host species, and county, I started by sorting the DNA and cataloging the available samples. I then processed the samples using nested polymerase chain reaction (PCR) in order to amplify the nymphal DNA to allow for the visualization of nymph samples positive for lyme disease. Nested PCR is done by completing two rounds of PCR, the first using outer primers and the second using inner primers, which provides a higher specificity and sensitivity for the samples ensuring that the results are accurate. Utilizing gel electrophoresis, I was able to identify the DNA samples positive for lyme disease.

To further analyze the data, I organized it by year, county, and host species using a contingency table in RStudio. The host species were categorized as “chipmunk”, “mouse”, “vole”, and “other”, with “other” category including less common host species like the Northern short-tailed shrew. The nymphs were collected from seven counties in Minnesota, including Anoka, Carver, Dakota, Hennepin, Ramsey, Scott, and Washington counties from 1991 to 2016, but only data from 1993 to 2016 was utilized in the statistical analysis. After the contingency table was created, I conducted a chi-square test of homogeneity with a significance level of 0.05. My null hypothesis was that the nymph host distribution is identical across all counties, while my alternate hypothesis was that the nymph host distribution is not identical across all counties. The chi-square test provided a p-value of 2.2e-16, which is smaller than the significance level of 0.05. Because the p-value is less than the significance level, the data provides evidence against the null hypothesis, meaning that we have statistically significant evidence that the nymph host distribution is not identical across all counties. Because some of the expected cell values were less than five, I also conducted a Fisher’s Exact Test based on 2,000 replicates resulting in a p-value of 0.0004998, providing further evidence against the null hypothesis allowing for its rejection.

To investigate the number of nymphs collected per year for each host type, I conducted Pearson correlation tests using year and the number of nymphs for each category. The correlation coefficients were 0.310, 0.643, 0.571, and -1 for “chipmunk”, “mouse”, “vole”, and “other” host categories, respectively. A positive correlation coefficient indicates that there is a positive linear relationship between the variables, with a larger number representing a stronger association. This means that over time, the frequency of ticks found on chipmunk, mice, and vole has increased, with mice and vole having a larger positive correlation indicating a moderate to strong relationship between the passage of time and frequency of nymphs found on mice and vole in the Twin Cities Metropolitan Area. The “other” category has a correlation coefficient of -1, indicating a strong inverse relationship between the passage of time and the frequency of nymphs found, but the sample size for this category was extremely small meaning this correlation is likely not statistically significant.

Discussion & Evaluation

Due to the utilization of a pre-existing collection of DNA, some limitations for the statistical analysis portion of this project were present. This includes the presence of cell counts less than five, which potentially introduced error into the chi-square p-value, the lack of data for the “other” category of host species, likely affecting the correlation test for that category, and the shortage of data from certain counties (e.g., Carver, Scott). Taking into account the available data, my objectives were accomplished for the statistical analysis. Furthermore, I think that I effectively processed the samples using PCR to the best of my abilities. Although I initially aimed to progress more quickly through this aspect of my project, there was a learning curve for this technique that I had to push past before becoming completely comfortable with the procedure and making faster progress.

My experience in the Undergraduate Research Opportunities Program (UROP) was a fantastic learning experience that I am extremely grateful and proud to have been a part of. I believe that the guidance I received through this program and from my mentors was a huge component of my success and helped me become much more confident and comfortable in the lab. I appreciated the structure and direction provided by UROP, as it helped me navigate my first exposure to lab work and opened the door to other opportunities.

Sources

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