

Reconstructing a Phylogenetic Tree of Local Tick Species Found on Pets

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Abstract:

With 300,000 cases of tick bites reported annually in the US, New Jersey holds one of the highest rates of Lyme disease through ticks. It is crucial to understand the biodiversity of ticks across New Jersey and New York, since each tick may carry an array of pathogens. To inform the public, we visualized the tick biodiversity and associated diseases through a phylogenetic tree. We collected 13 ticks from veterinary clinics. The DNA Extraction, Polymerase Chain Reaction (PCR), Gel Electrophoresis, and DNA Sequencing of our samples were performed by a scientist at Cold Spring Harbor Laboratories. We used DNA Subway software to analyze and trim DNA for quality control, BLAST software to identify the species and create a phylogenetic tree, and the CDC “Diseases Transmitted by Ticks” Database to identify associated diseases. We observed that 100% of our samples were *Ixodes Scapularis*, representing its high prevalence in our region.

Introduction:

It is estimated that from 1% to 50% of ticks carry diseases, many of which are life-threatening [7]. These ticks latch onto pets and humans, and even though many humans utilize various preventions for themselves and their animals, 300,000 cases of tick bites are reported per year [1]. New Jersey has one of the highest rates of Lyme disease in the USA. In 2018, out of the 33,666 cases of Lyme disease, New Jersey held 11% of these cases [6]. Although 33,666 cases of Lyme disease may seem insignificant to the 382 million Americans out there, the CDC estimates that 300,000 cases of Lyme disease are more accurate, as a majority of the cases go unreported. In addition, Lyme disease is not the only tick-borne disease out there [2]. The CDC lists 16 different diseases that are transmitted by ticks and Lyme disease is only a part of the larger pool. The number of cases of tick-borne diseases in 2018 was 47,743 but this number is rapidly increasing [10].

Ticks, although small and unnoticeable, pose a high danger to both humans and pets alike. They are parasitic arachnids that feed on the blood of their hosts, which are typically humans and other animals [12]. In addition to the fact that ticks spread diseases to humans, it is crucial to understand the range of tick species across New Jersey and New York as each tick can carry a wide array of bacteria, spirochetes, rickettsiae, protozoa, viruses, nematodes, and toxins [9]. Currently, the only preventative measures against ticks are avoiding areas of high congestion of ticks and using repellent. There is currently no vaccine for tick-borne diseases. To alleviate this issue, it is important to have a method to efficiently visualize what ticks are in someone's local area and more importantly, what diseases those ticks commonly transmit.

The recent increase in ticks across the United States only means that it is important to track the ancestry of these tick species that are found in our town. These ticks are ubiquitous in local neighborhoods and our research will hopefully contribute to keeping our pets and families safer. Thus, we identified a number of tick species, replicated its phylogenetic tree, and pinpointed potential pathogens that are linked to the specific species. We focused our sample

collection on ticks found on household pets that are in approximately 65% of all American households [9]. Through reconstructing a phylogenetic tree, we tracked the ancestry of potential pathogens that are associated with the tick species that have been found in pets in New Jersey and New York and examined the biodiversity of our samples to the data of Pennsylvania Tick Labs.

Materials and Methods:

First, we collected 13 tick samples from veterinary clinics. Only 3 samples were viable to follow through our DNA Extraction. For each sample, we documented which animal hospital it was collected from, an image of each sample, and labelled each sample with a number between one and thirteen.

Next, each sample was in a refrigerated environment in a freezer at our homes. We inputted all data into the Cold Spring Harbor DNA Learning Center Barcode Sample Database.

We were unable to perform DNA Extraction ourselves, so we sent our samples to the Cold Spring Harbor Laboratory where a scientist extracted the DNA for us. This process was as follows.

For DNA extraction, a piece of tissue of each sample was placed in a respective 50 μ L lysis solution. A pestle was used to grind the tissue into particles in the lysis solution. A 3-mm diameter disc of Whatman No. 1 Chromatography paper was added to the lysed extract. The tube was flicked so that the disc was in the extract. The disc soaked for one minute. After one minute, the disc was transferred to a tube containing a 200 μ L wash buffer. The disc in the tube was mixed for 5 seconds, and then left to settle for 1 minute. This tube was then labeled with the sample number.

The Whatman disc was then transferred onto a square of foil labeled with the sample number. After drying for 10 minutes, the foil was folded and sent to DNALC. for PCR and Gel Electrophoresis.

The Polymerase Chain Reaction and Gel Electrophoresis was also conducted by a scientist at the Cold Spring Harbor Laboratory due to COVID-19.

DNA Subway was used to analyze and trim the DNA sequence for quality control. DNA Segments with the highest quality were selected, and lower quality DNA was trimmed. BLAST was used to compare the DNA of the samples to known DNA databases in order to identify the species of tick, and we constructed a phylogenetic tree of the tick samples provided. The CDC “Diseases Transmitted by Ticks” Database [4] was used in order to identify possible diseases that are found in the tick species. Data collected from Pennsylvania Tick Labs [11] was used to compare our samples with.

Results:

Our data revealed that Samples 3, 5, and 6 out of our original 13 samples were identified as the species *Ixodes Scapularis* as their sequences are the most similar. Our phylogenetic tree can be seen below in Figure 1.

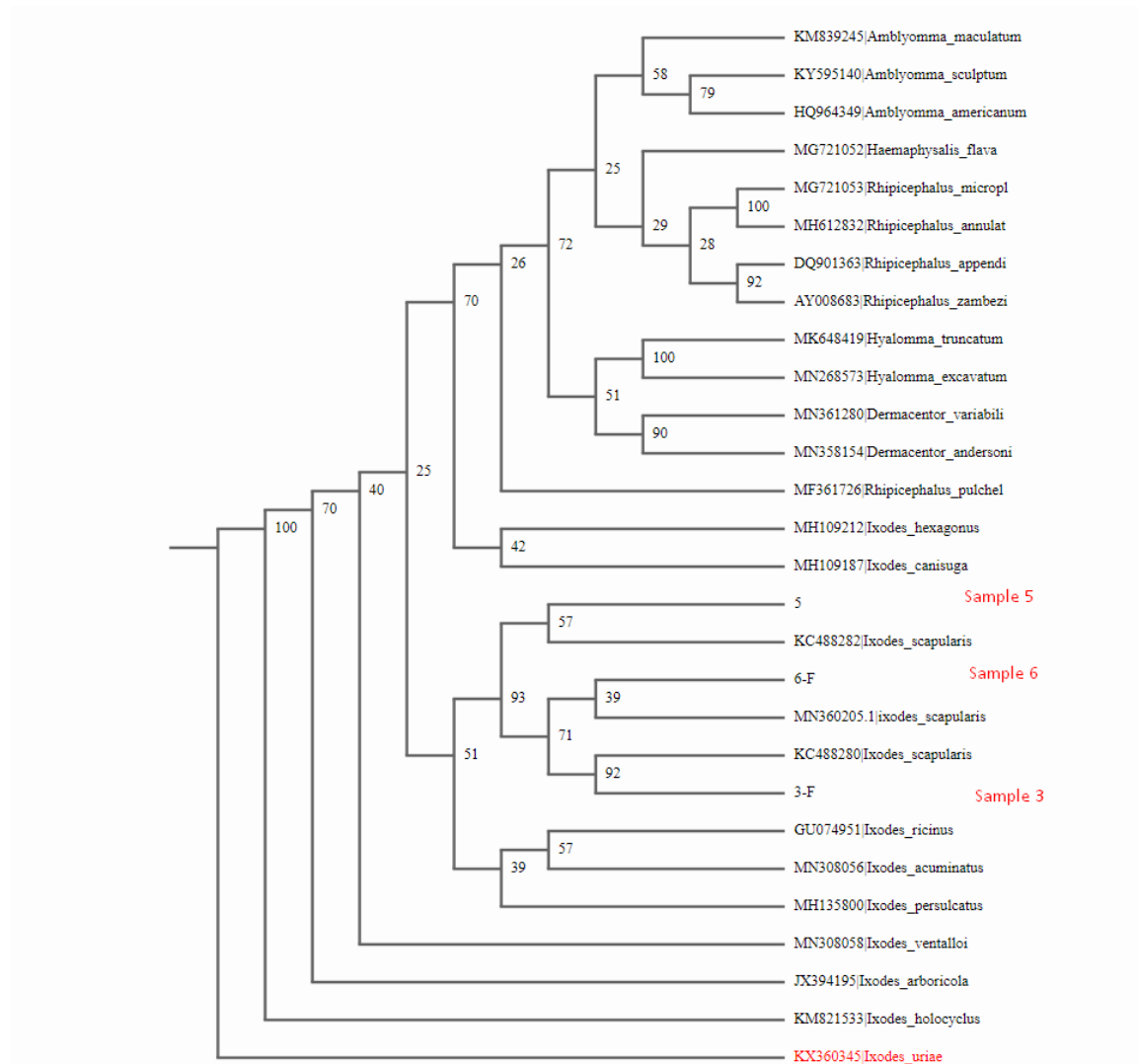
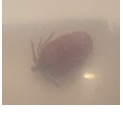


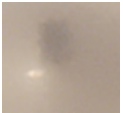
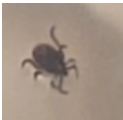
Figure 1: Phylogenetic tree of the 13 original samples.

In addition, Table 1 details the associated diseases between each of our tick samples.

Table 1: Table detailing the sample number of each tick, the species it was found on, the identified species name, the related diseases to the species, the veterinary clinic that provided the tick, the date the tick was collected, and a photo of each sample, from left to right.

Sample #	Pet Species	Identified Tick Species	Related Diseases	Veterinary Clinic Provided	Date sample was received	Sample Image

Sample #1	Dog	Not identified	N/A	Bergen Veterinary	01/04/21	
Sample #2	Cat	Not identified	N/A	Bergen Veterinary	01/04/21	
Sample #3	Dog	Ixodes Scapularis	Lyme Disease, anaplasmosis, babesiosis, borrelia myamotai disease, powassan virus disease, ehrlichiosis	Cresskill Animal Hospital	01/05/21	
Sample #4	Dog	Not identified	N/A	Loving Care Hospital	02/05/21	
Sample #5	Dog	Ixodes Scapularis	Lyme Disease, anaplasmosis, babesiosis, borrelia myamotai disease, powassan virus disease, ehrlichiosis	Loving Care Hospital	02/05/21	
Sample #6	Dog	Ixodes Scapularis	Lyme Disease, anaplasmosis, babesiosis,	Loving Care Hospital	02/05/21	

			borrelia myamotoi disease, powassan virus disease, ehrlichiosis			
Sample #7	Dog	Not identified	N/A	Loving Care Hospital	02/05/21	
Sample #8	Dog	Not identified	N/A	Loving Care Hospital	02/05/21	
Sample #9	Dog	Not identified	N/A	Loving Care Hospital	02/05/21	
Sample #10	Dog	Not identified	N/A	Loving Care Hospital	02/05/21	
Sample #11	Dog	Not identified	N/A	Loving Care Hospital	02/05/21	
Sample #12	Dog	Not identified	N/A	Loving Care Hospital	02/05/21	
Sample #13	Dog	Not identified	N/A	Loving Care Hospital	02/05/21	

Discussion:

While conducting this research, we encountered a few issues. Our first issue was that out of safety concerns, our ticks were sourced from veterinary clinics rather than the wild, which led to us collecting a fewer amount of ticks as our total sample. As well, due to the COVID-19 pandemic, we were unable to perform the DNA extraction, Polymerase Chain Reaction, Gel Electrophoresis and DNA Sequencing ourselves, but a scientist at Cold Spring Harbor Laboratory graciously helped us by performing these processes for us. Lastly, only 3 out of our 13 collected samples were viable in the DNA sequencing lab at this current time. Due to the low amount of samples we were able to collect, we analyzed external data to compare our results with more general results from a larger institution, Tick Research Lab of Pennsylvania. This institution provides their information from ticks collected from all over the United States and their own laboratories to identify what pathogens reside on each tick. The biodiversity of our sample is that in our local area, we found 100% prevalence of *Ixodes Scapularis* (Deer Tick). Although this is not representative to the true local biodiversity, it demonstrates that this specific species of ticks holds significant prevalence in our region. To support this, the Tick Research Lab of Pennsylvania has compiled national and state-wide data on tick statistics. They found that in their samples, they had approximately 62.2% of their samples as *Ixodes Scapularis* (Deer Tick) [10]. The associated diseases to this species are Lyme disease, Anaplasmosis, B. miyamotoi disease, Ehrlichiosis, Babesiosis, Powassan virus disease. This indicates that it is important to be aware of these diseases when walking around in a tick-prone area, especially during the months of high activity, such as during the spring and summer months. In conclusion, the issue that we attempted to tackle is that there is a high, increasing prevalence of tick species that transmit pathogens in which humans have no vaccine or substantive preventatives against. To establish the regional risks to our community, we identified local tick species and presented our findings in a phylogenetic tree. We successfully created this tree and discovered that 100% of our samples were *Ixodes Scapularis* (Deer Tick), which is what the Tick Research Lab of Pennsylvania's data support as they too present data with a high prevalence of this species in the NJ and NY region. For future research, the direction could take the approach of examining a wider range of samples and determining the pathogens found on the samples to conclusively determine the risks that specifically pertain to our region to confirm our observations.

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