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Testing Tick Repellent Effectiveness

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Background

- Ticks are a species of arthropod and vector of disease. Ticks viruses, protozoa, and bacteria *Rickettsia*, which causes Rocky Mountain Spotted Fever, and Spirochetes, which causes Lyme disease.
- In Washington State, the most common tick species are (Figure 1):
  - *Dermacentor variabilis* (American Dog tick)
  - *Dermacentor andersoni* (Rocky Mountain Wood tick)

Figure 1

Female and male *Dermacentor variabilis* (American Dog tick). https://tickencounter.org/tick_identification/tick_species

Female and male *Dermacentor andersoni* (Rocky Mountain Wood Tick) https://tickencounter.org/tick_identification/tick_species

- A tick has four life stages, including egg, larva, nymph and adult (Figure 2). Once it hatches, the tick requires blood from a host to proceed to the next phase.
- Ticks feed through a process called questing, a sit-and-wait approach conducted to enhance the likelihood of finding a host.
- Prey is often detected via body odor, body temperature, and vibration's from moving past.
- Strategies to prevent being bitten and exposed to potentially tick-borne disease include: protective clothing, repellents, and staying out of tick prone areas.
- Repellents do not work like insecticides that kill the target; they help prevent contact. Between the host and vector.
- There are homeopathic and chemical-based repellents available. Both help prevent tick bites, but homeopathic options do not have to be registered by the Environmental Protection Agency.
- Three important reasons to use tick repellent are:
  1. There is an emerging threat to human health by tick-borne disease
  2. There are dominant insect vectors of disease in new regions
  3. It is too difficult to prevent disease from arthropods by using only vaccinations

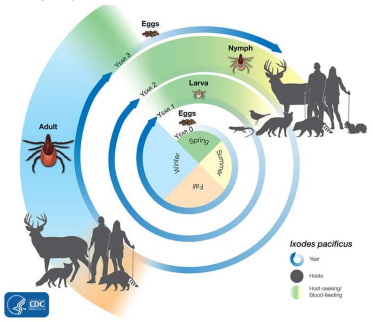

Figure 2: The life cycle of *Ixodes pacificus* ticks over the course of three years. Demonstrates during which seasons, this species is most likely to seek and feed upon a host. Throughout each stage, the size of the host becomes slightly larger than the previous (How ticks spread disease | Ticks | CDC).

Objective

Ticks are responsible for transmitting at least 14 known pathogens in the United States and ticks positive for disease have been identified at multiple locations within the state of Washington (Figure 3). Therefore, testing these three repellents will be beneficial from a public health and disease ecology standpoint.

Figure 3: A visual of Washington State and the areas where ticks were collected as well as tested for pathogens (Massart, 2019).

The objective of this study is to test which of the following name brand repellents will be the most effective in reducing tick-borne illnesses and detouring ticks from individuals' bodies.

- Off! Deep Woods Insect Repellent VII
- Repel Plant-Based Insect Repellent lemon eucalyptus Insect Repellent
- Sawyer Picaridin Insect Repellent

Statistical Analysis

- Our hypothesis is "If we use a repellent that has a higher concentration of DEET compared to a lower or no concentration, then the repellency against ticks will be significantly greater".
- To analyze our numerical data, we will be using a programming language called R for statistical computing accompanied by RStudio (IDE). We will input our data into R using a series of codes to generate graphs for visual representation of each repellent used as well as test our data for normality using Gaussian distribution.
- We will also take our data and calculate the p-value ($p<0.05$) to determine whether there is a significant difference between repellent efficiency. Calculating the p-value will represent the probability of obtaining a result/results that are as extreme as the observed results of our statistical analysis, assuming that the null hypothesis is correct.
- With explanatory variables being types of repellents used and the response variable being the amount of time a tick spends on a treated surface, we will use Poisson regression to compare the times spent. This comparison will allow us to see which repellent has a statistical significant effect on the response variable therefore allowing us to accept or decline the null hypothesis.

Methods and Procedures

The methods of this experiment are based on the procedures set forth by Semmler et al in their 2011 study titled "Comparison of the tick repellent efficacy of chemical and biological products originating from Europe and the USA".

Collection:

- Each researcher will capture between 20 and 25 ticks and transfer them using forceps/tweezers into a glass mason jar fitted with a fine mesh covering over the opening, secured with a mason jar ring, and containing wet cotton balls for moisture.
- At the conclusion of the study, all captured ticks will be placed in vials containing ethanol, noting collection date and site, and shipped to Dr. Magori for his continuing research.
- All aspects of the study will be performed individually, by each researcher, at various locations and data will be pooled for analysis.
- Researchers will follow appropriate safety precautions as outlined by Dr. Magori.
- GPS coordinates of each tick collection site will be obtained by phone or Google maps and pictures of those sites will be taken as well.
- To simulate the movement of animals or humans, a corduroy blanket will be dragged for 50 meters through the potential tick habitat. The blanket will then be inspected and any "captured" ticks will be removed and placed in the jar.
- Collected ticks will also be identified using the online identification key provided by the University of Rhode Island at www.tickencounter.org

Testing:

- Testing will take place indoors, at room temperature. Widows will be closed and ventilation turned off to avoid air movement disruption of tick behavior.
- After collection, the corduroy blanket used to capture ticks will be shaken to loosen any debris, and cut into 4 equal 18 x 24 inch pieces as shown in Figure 4.
- Each blanket piece will be labelled in the corner with a number from 1 to 4 using a sharpie and a 5 cm diameter circle will be drawn in the center as a treatment application target and for tracking tick movement.
- Prior to treatment of blanket, test liquids will be transferred from their original container to 2 oz glass spray bottles each labeled 1 to 4
- Labelled blanket pieces will be treated in the circle with the following substances:
  - 1 = Tap water (control treatment)
  - 2 = Off! Deep woods Insect Repellent VII
  - 3 = Repel Insect Repellent (Plant-Based, Lemon Eucalyptus)
  - 4 = Sawyer Picaridin Insect Repellent

Expected Results

- The premise of this experiment is to identify which tick repellent performs best in deterring ticks from humans.
- Our belief is that there will be a significant difference between the type of repellent and its effectiveness.
- More specifically, we predict that the repellent containing DEET will be the most effective at repelling ticks.
- If the data demonstrates there is no statistically significant difference among repellent types, then we will have to fail to reject the null hypothesis that there is no statistical significance between type of tick repellent and efficacy.

Methods and Procedures cont.

- Treatment application:
  - Blanket piece will be held vertically in one hand and its matching spray bottle will be held in the other.
  - The spray bottle will be brought 6 inches from the center of the circle drawn on the blanket piece surface and pumped 1 time.
  - Process will be repeated for each of the 4 treatment groups.
  - Liquid will be allowed to dry for approximately 5 minutes prior to tick placement
- Tick response testing:
  - Blanket piece will be laid horizontally on a surface and a single tick will be placed in the center of the drawn circle.
  - Ten seconds after tick placement, the top 4 inches of the blanket piece will be secured to a tabletop with a heavy object (e.g., textbook) and the rest will be allowed to freely hang from the tabletop edge as shown in Figure 4.
  - A box or a bin will be placed on the ground underneath the hanging blanket piece to catch any dropping ticks.
  - The test trials will run for 2 minutes.
  - Each researcher will replicate this process 5 times for each treatment group, resulting in a total of 20 tests performed by each researcher.
- Potential Results:
  - Positive (indicating repellent worked) à Tick drops from blanket piece within 2 minutes
  - Negative (indicating repellent not effective) à Tick climbs upward at least 5 cm within 2 minutes
  - Inconclusive à Tick remains stationary (fails to drop or climb the minimum 5 cm)
- Ticks will not be reused in this experiment. A new tick will be used for every replication to avoid testing bias and to minimize harm since it is assumed that repellents are irritative to ticks.
- After researchers have completed their individual 20 tests, they will input their data to a shared online document (see table 1) and data analysis will be performed on the pooled results.

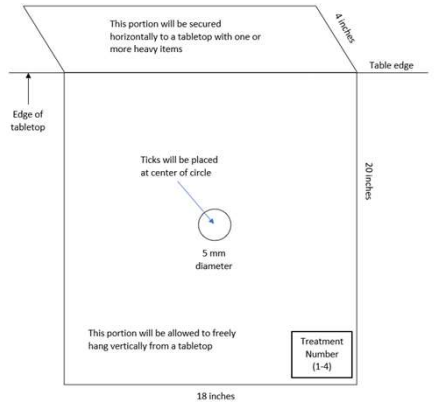

Figure 4: A visual representation of the corduroy blanket pieces that will be used for testing repellents.