The Interaction between Species and *Rickettsia* Presence Influences Tick Gut Microbial Communities

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Introduction

- *Amblyomma americanum, Amblyomma maculatum*, and *Dermacentor variabilis* are the most commonly encountered ticks in the southeastern United States. These ticks are of medical and veterinary importance, as they can be vectors for human and animal-based pathogens.

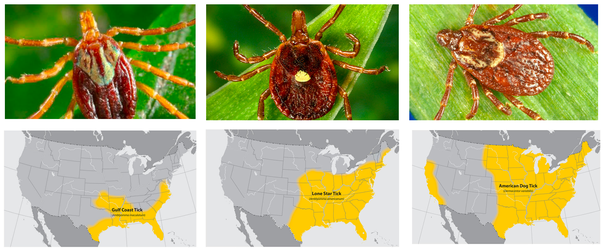

Fig. 1: Comparison of the habitat ranges for *Amblyomma americanum*, *Amblyomma maculatum*, and *Dermacentor variabilis*. (Credit: CDC)

- Spotted fever group Rickettsiosis (SFGR) is a class V epizootic with multiple vectors, reservoirs, and pathogens (Mather & Ginsberg 1994)
- One pathogen of particular interest within this group is *Rickettsia rickettsii*, which can cause Rocky Mountain spotted fever (RMSF), a potentially life-threatening disease in humans that can be found in both *A. americanum* and *D. variabilis*
- Other pathogenic *Rickettsia* include *R. parkeri, R. amblyommatis,* and *R. montanensis,* and which are found within all three tick species
- Previously, Trout Fryxell and DeBruyn (2016) reported microbial differences in *A. americanum* collected from different habitats
- Our goal was to understand the bacterial communities within three tick species as they relate to the presence and absence of *Rickettsia*, and to investigate reasons for potential differences and/or inconsistencies

Methods

- Specimens were selected from previously collected ticks. These ticks were collected from May to August of 2012 from the Ames Research & Education Center in western Tennessee and screened for the detection of the *ompA* protein in *Rickettsia* species using PCR (Trout Fryxell et al. 2016).
- A total of 184 specimens were selected consisting of 24 *A. americanum* (6 positive and 18 negative), 92 *A. maculatum* (24 positive and 68 negative), and 75 *D. variabilis* (25 positive and 50 negative).

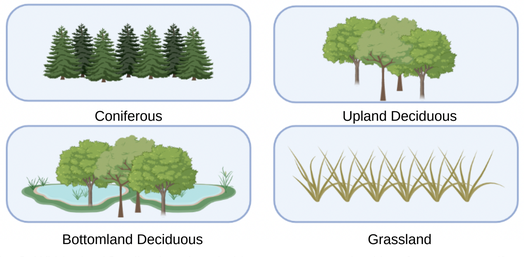

Fig. 2: Within the 13 collection sites, habitats were categorized into four types: coniferous, bottomland deciduous, upland deciduous, and grasslands (Credit: Biorender)

- Illumina sequencing of 16S rRNA gene amplicons for each specimen and subsequent analyses was performed with the 'dada2' package (version 3.15)
- Libraries that contained ASVs classified as *Rickettsia* were categorized as 'ASV-positive' and those that did not were 'ASV-negative'.
- Statistical analysis was conducted relating richness and diversity to species, *Rickettsia*, habitat, and sex.
- Further analysis compared ticks that were both PCR-positive and ASV-positive (+/+) with those that were both PCR-negative and ASV-negative (-/-).

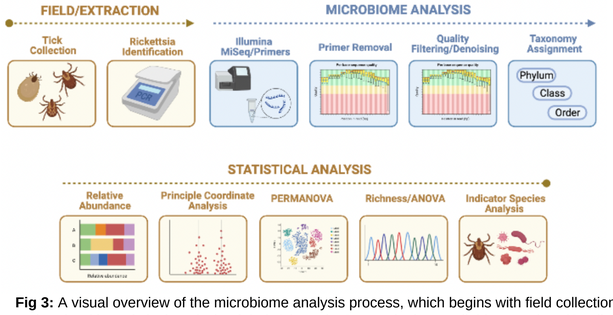

Fig 3: A visual overview of the microbiome analysis process, which begins with field collection and ends with statistical analysis of dada2-based ASVs and other available metadata (Credit: Biorender).

Results

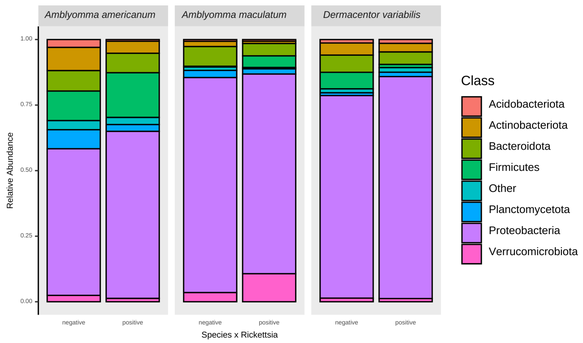

Fig 4: An abundance chart representing the biodiversity of bacteria in adult *A. americanum, A. maculatum,* and *D. variabilis*. Diversity was stratified by both species and *Rickettsia (*PCR-test results). In all species Proteobacteria dominated the bacterial composition, although *A. americanum* had a higher presence of other bacteria than *A. maculatum* and *D. variabilis.*

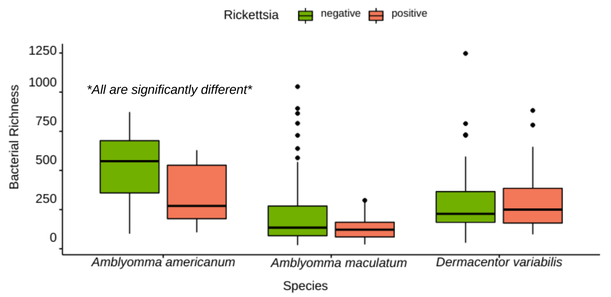

Fig 5: Boxplot comparing bacterial richness with tick species x *Rickettsia* detection. We found that *A. americanum* had a higher median richness in *Rickettsia* positive samples than the remaining treatment groups. Additionally, bacterial richness differed significantly by tick species ($X2$ = 4187.6; df = 2; $P < 0.001$) and whether *Rickettsia* was detected ($X2$ = 378.2; df = 1; $P < 0.001$). Furthermore, a significant interaction ($X2$ = 663.7; df = 2; $P < 0.001$) showed that the bacterial richness of the two *Amblyomma* species responded the same (decreasing with positive *Rickettsia* infection), while *D. variabilis*, increased in gut bacterial richness when *Rickettsia* was detected.

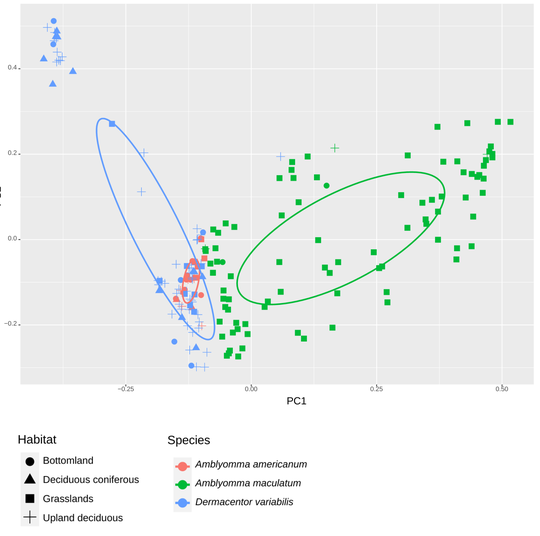

Fig 6: A principal coordinate analysis (PCoA) figure using the Bray-Curtis method to assess the differences and similarities between species and habitat. We found that the bacterial composition *D. variabilis* and *A. maculatum* were significantly different from one another (F = 11.719; df = 2; $P < 0.001$). Furthermore, the bacterial composition of *A. americanum* was more similar to *D. variabilis* than *A. maculatum.* We then looked at the habitat type by tick species and found that the bacterial composition also differed (F = 5.4277; df = 3; P<0.001) based on which habitat the ticks were sampled from (indicated by the shapes).

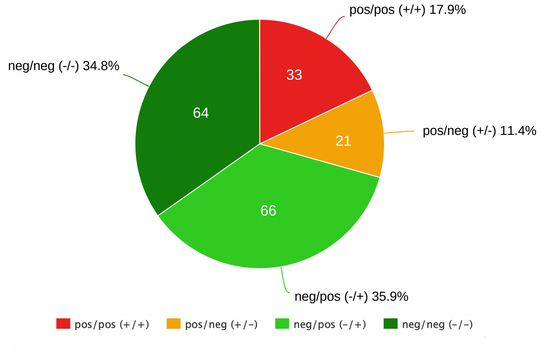

Fig 8: Overview of the testing results for *Rickettsia* detection. Results were categorized by true positive (+/+), PCR-positive but ASV-negative (+/-), PCR-negative but ASV-positive (-/+), and true negative (-/-). A contingency test compared the two assays and indicated results were not significantly different ($X2$ = 1.6417, $P > 0.05$).

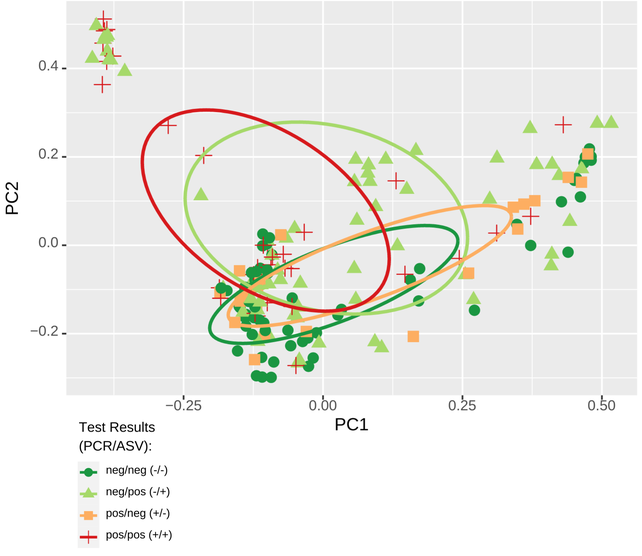

Fig 7: Another PCoA was created to assess bacterial structural differences between the two different *Rickettsia* detection results. Results were categorized by true positive (+/+; plus), PCR-positive but ASV-negative (+/-; square), PCR-negative but ASV-positive (-/+; triangle), and true negative (-/-; circle). The slight overlap of (+/+) and (-/+) indicates little bacterial compositional change between ticks with *Rickettsia* that were detected through PCR and those that were not. Overall results show that bacterial composition between samples df does not change if *Rickettsia* is present or not.

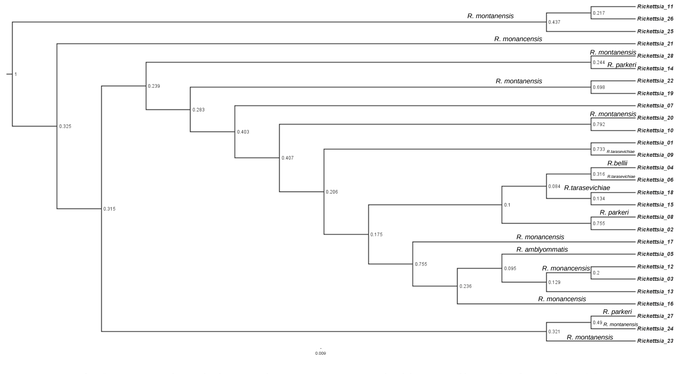

Fig 9: A Bayesian phylogenetic tree was created with Beauti/BEAST from the 16S *Rickettsia* ASV's present in the samples. Sequences were identified with NCBI BLAST. Of the results, there was identified sequences for *R. amblyommatis*, *R. belli*, *R. monancensis, R. montanensis*, *R. parkeri,* and *R. tarasevichiae.* Note, ASV sequences for Rickettsia_11, Rickettsia__14, Rickettsia_15, and Rickettsia_17 were ~100bp shorter than the rest which may have affected identification. Posterior probability values are provided at branch nodes to indicate degree of relatedness (values closer to 0 are more similar).

Conclusions

The presence of *Rickettsia* **changed bacterial richness** in the **tick gut microbiome**

- In all tick species Proteobacteria dominated the bacterial composition, with *A. americanum* containing the most evenness between the different bacterial classes.
- Regardless of PCR or ASV analysis detection, the presence of *Rickettsia* did not change bacterial composition; however, PCR accuracy needs further analysis.

High variation in **gut bacterial composition** is driven by a number of factors, including tick **species** and **habitat**.
Tick sex did not influence bacterial composition.

- The bacterial composition of each tick species was found to be different from one another
  - Interestingly, the composition of *A. americanum* and *D. variabilis* were more similar to one another than *A. americanum* and *A. maculatum* which suggests that bacterial composition is not influenced by species phylogenetic relatedness and is more dependent on the ecology of each tick species.
- Twenty-eight unique *Rickettsia* ASVs were identified and those were similar to six different *Rickettsia* species.

Future Work

- Further analysis of *Rickettsia* detection methods is needed to determine the accuracy of PCR-testing:
  - Is the species of *Rickettsia* or amount of *Rickettsia* present in the sample related to the accuracy of the PCR-test?
  - Are there potential indicator species influencing the accuracy of PCR testing?
- Knowing six different *Rickettsia* were identified, our next step is to assess if any specific species contributed to additional variation.
- Habitat had an impact on bacterial composition; thus, further emphasis should be put on comparing the microbial communities of ticks as they relate to host choice and habitat use. This research includes looking into the entire microbial communities of each tick (including the fungal, protozoal, and viral microbes) collected from different hosts and habitats.

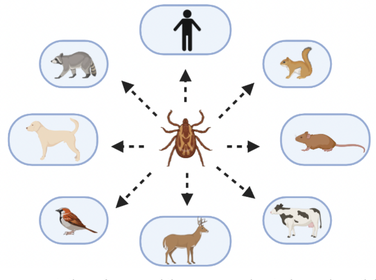

Fig 10: Visual representation of some of the common hosts for various tick species.

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