Microbial Community Assessment of Lone Star Ticks from Athens, GA

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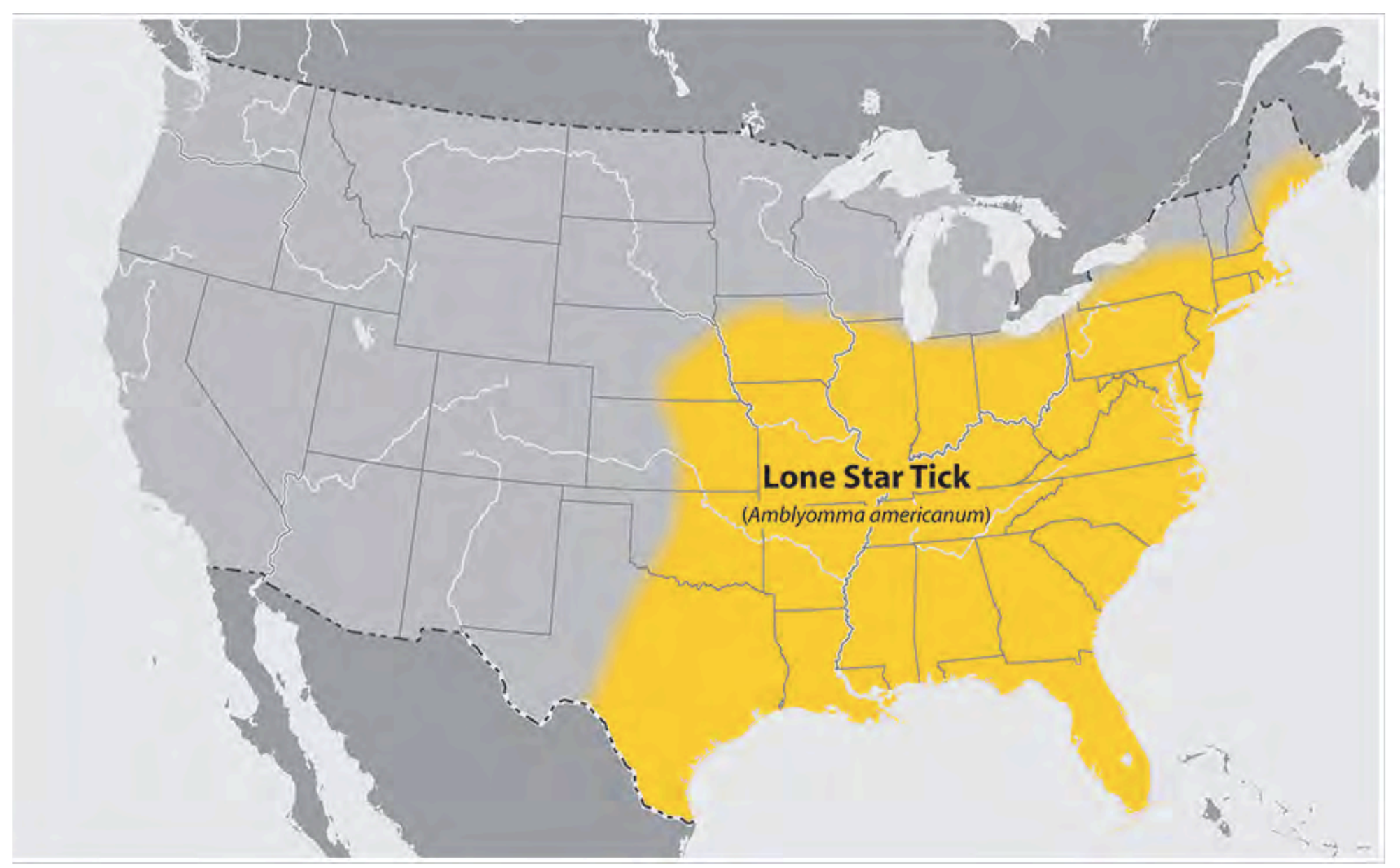
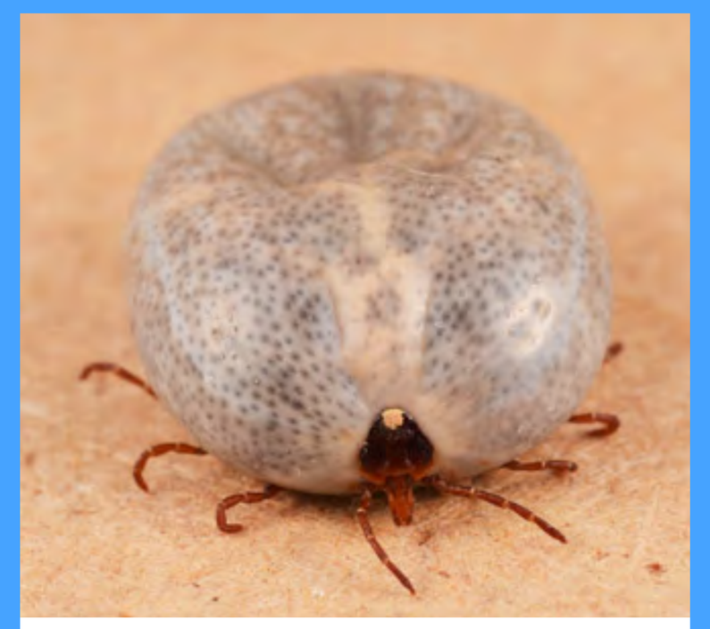


Figure 1[1]: Lone Star tick (*Amblyomma americanum*) distribution in the United States

A questing female lone star tick[2]

A male lone star tick[3]



A fully engorged female lone star tick[4]

Research Questions

- Do male and female Lone Star ticks share the same microbial communities?
- Does the Taq DNA polymerase used make a difference in the community amplified?

Introduction

- *Amblyomma americanum* (Lone Star) ticks are an important vector in the spread of disease, and cause millions in damage per year to livestock in Mexico.
- Lone Star ticks prefer mammalian hosts and spread several diseases including ehrlichiosis, babesiosis, Q fever, and rickettsial diseases (Goddard and Varela-Stokes 2008).
- These diseases pose a significant risk in terms of difficulty of diagnosis and treatment protocol.

Methods

Ticks were collected by dragging a white sheet through a wooded property on Simonton Bridge Road on two days in June, one week apart. Collected ticks were kept in ethanol for preservation. Ticks were identified with a dissecting microscope.

Ten male and ten female ticks from each collection were analyzed. DNA was extracted using Qiagen Tissue & Blood kits (Method 1 of Halos et al. 2004).

Tick DNA was then analyzed through Polymerase Chain Reaction (PCR) with Kappa HiFi HotStart or Kappa Robust Hotstart and broad spectrum 16S primers (Klindworth et al. 2013). The amplified DNA was sequenced using Illumina MiSeq v3 600 cycle kits to obtain paired-end 300 base reads.

DNA sequences were analyzed using Geneious R8. Reads were paired, trimmed, merged using FLASH, the community described via the 16S BioDiversity tool (Figs 2-4), then assembled if ≥98% similar and identified to species via BLAST.

Results

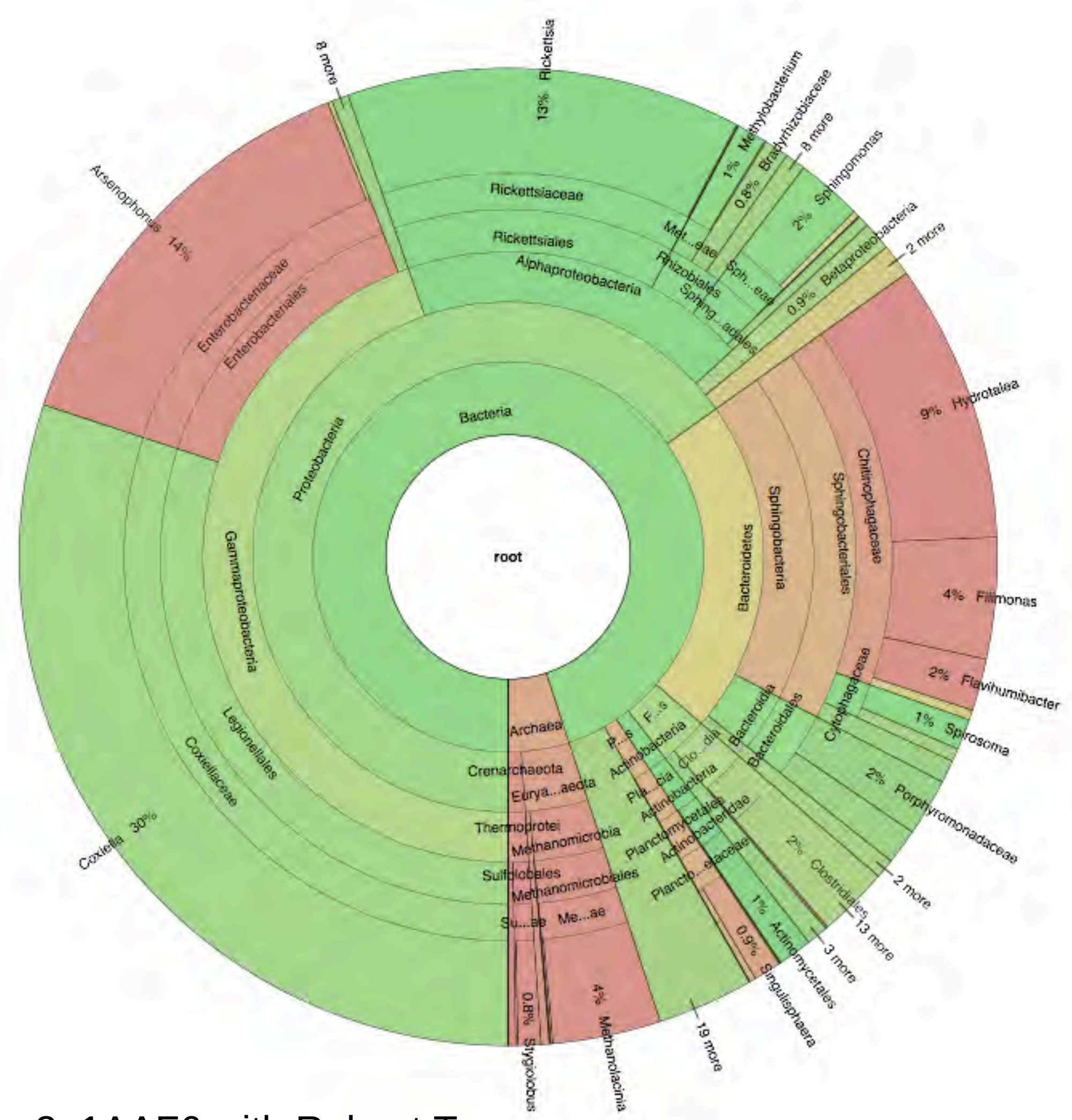


Figure 2: 1AAF6 with Robust Taq

Figure 3: 2AAM2 with Robust Taq

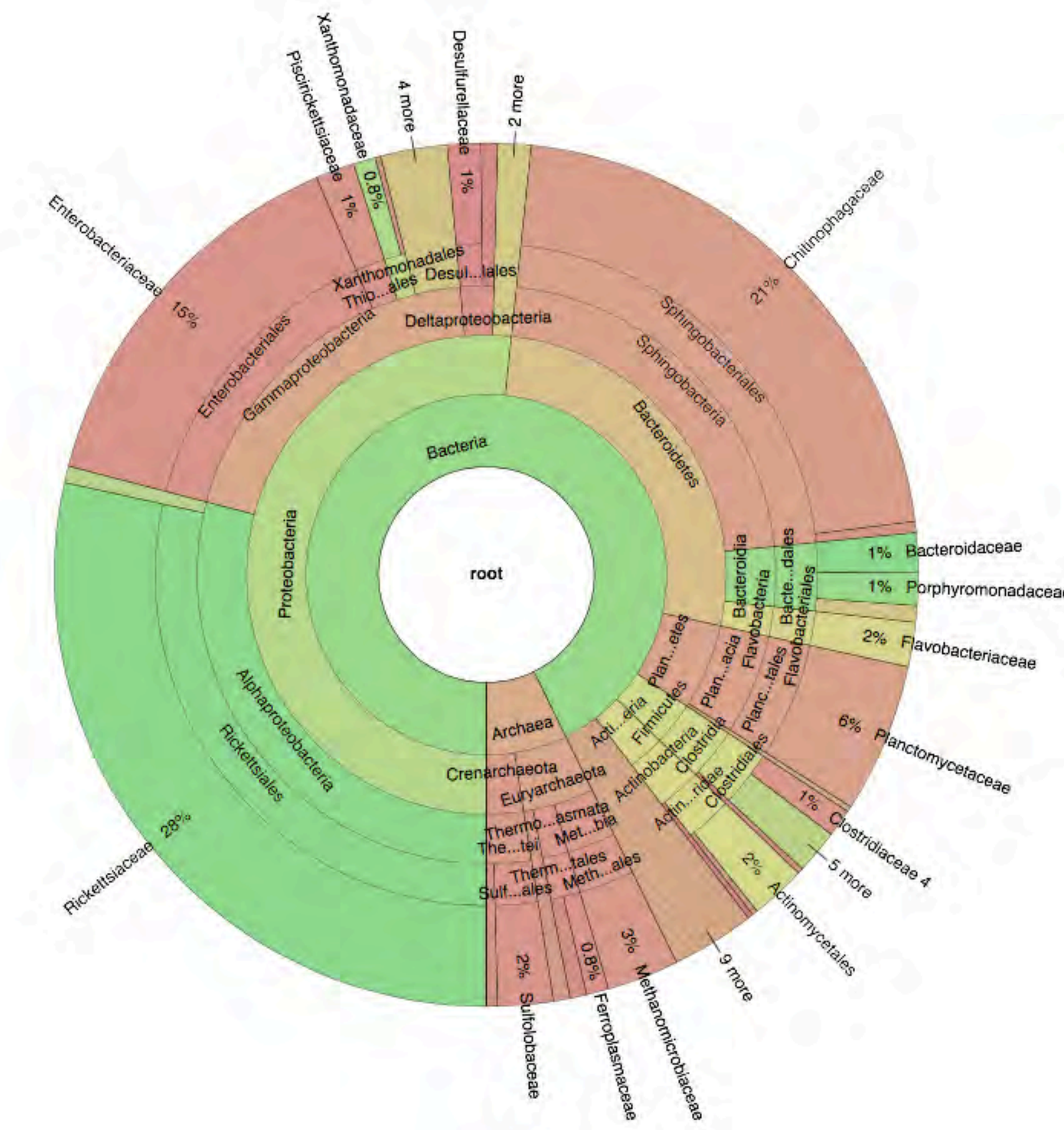
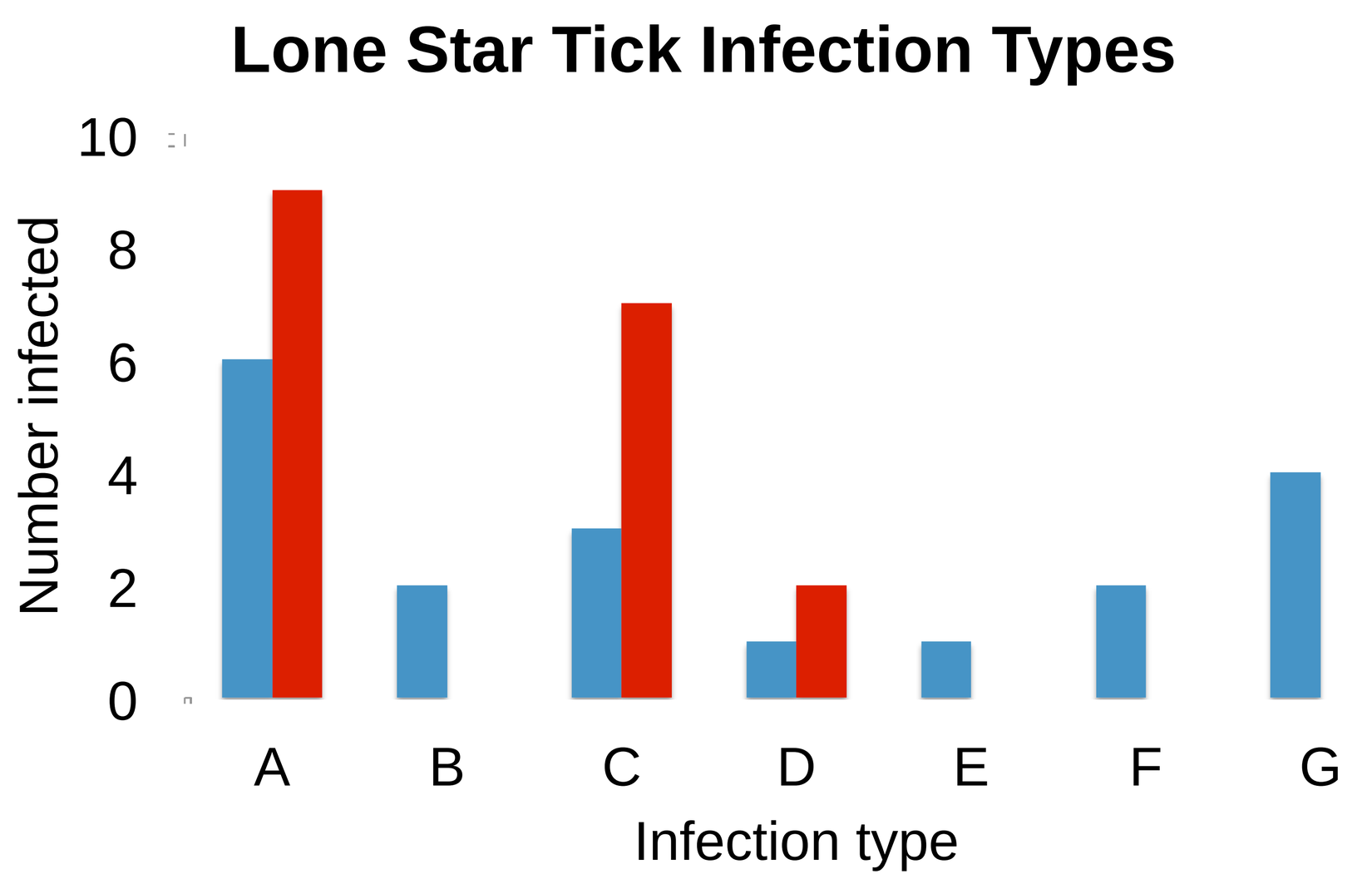
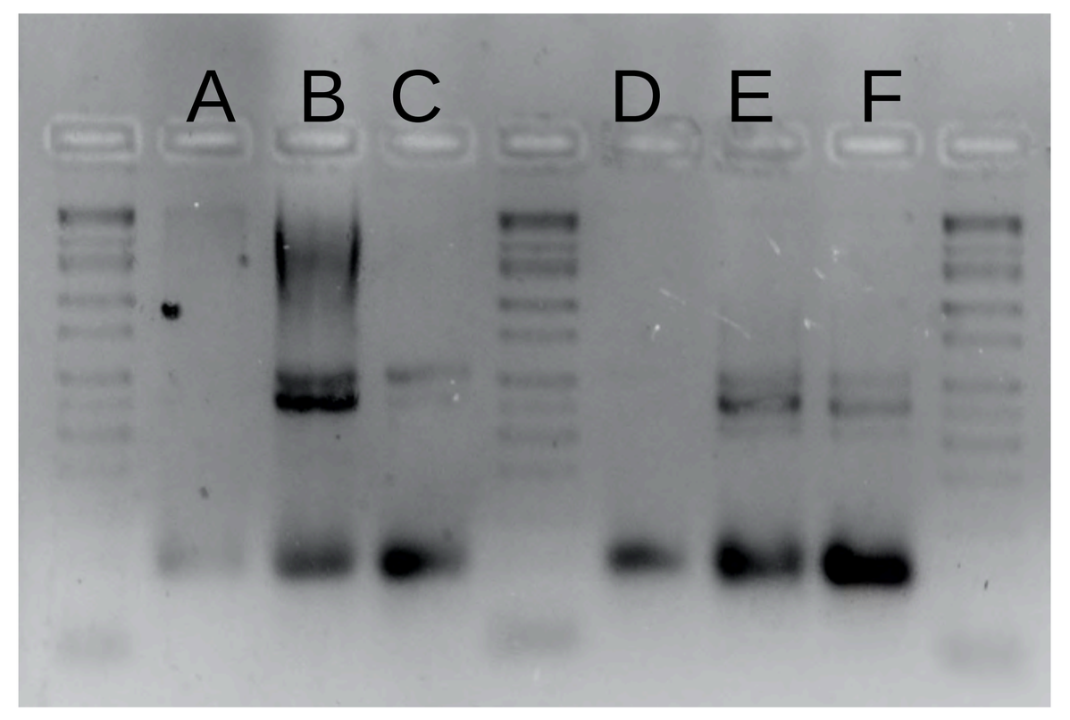


Figure 4: 2AAM2 with HiFi Taq



Legend	Fisher Exact Test Values[x]
A-*Coxiella*	0.32
B-*Rickettsia*	0.48
C-*Coxiella* and *Rickettsia*	0.15
D-*Coxiella* and *Ehrlichia canis*	0.60
E-*Rickettsia* and *Ehrlichia canis*	1.00
F-*Rickettsia* and *Ehrlichia chaffeensis*	0.48
G-No infection	0.11

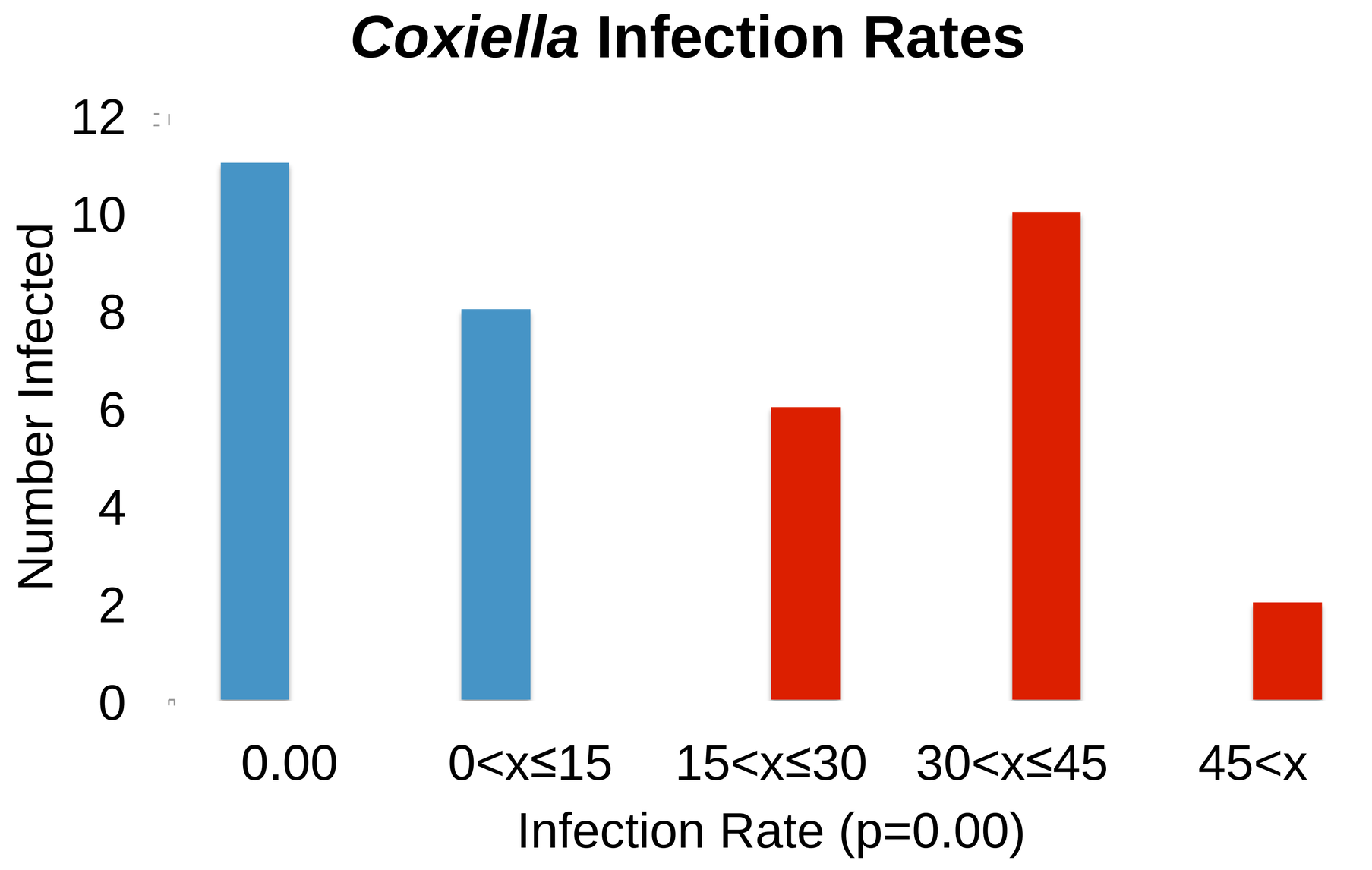
[x]Did not correct for multiple tests.

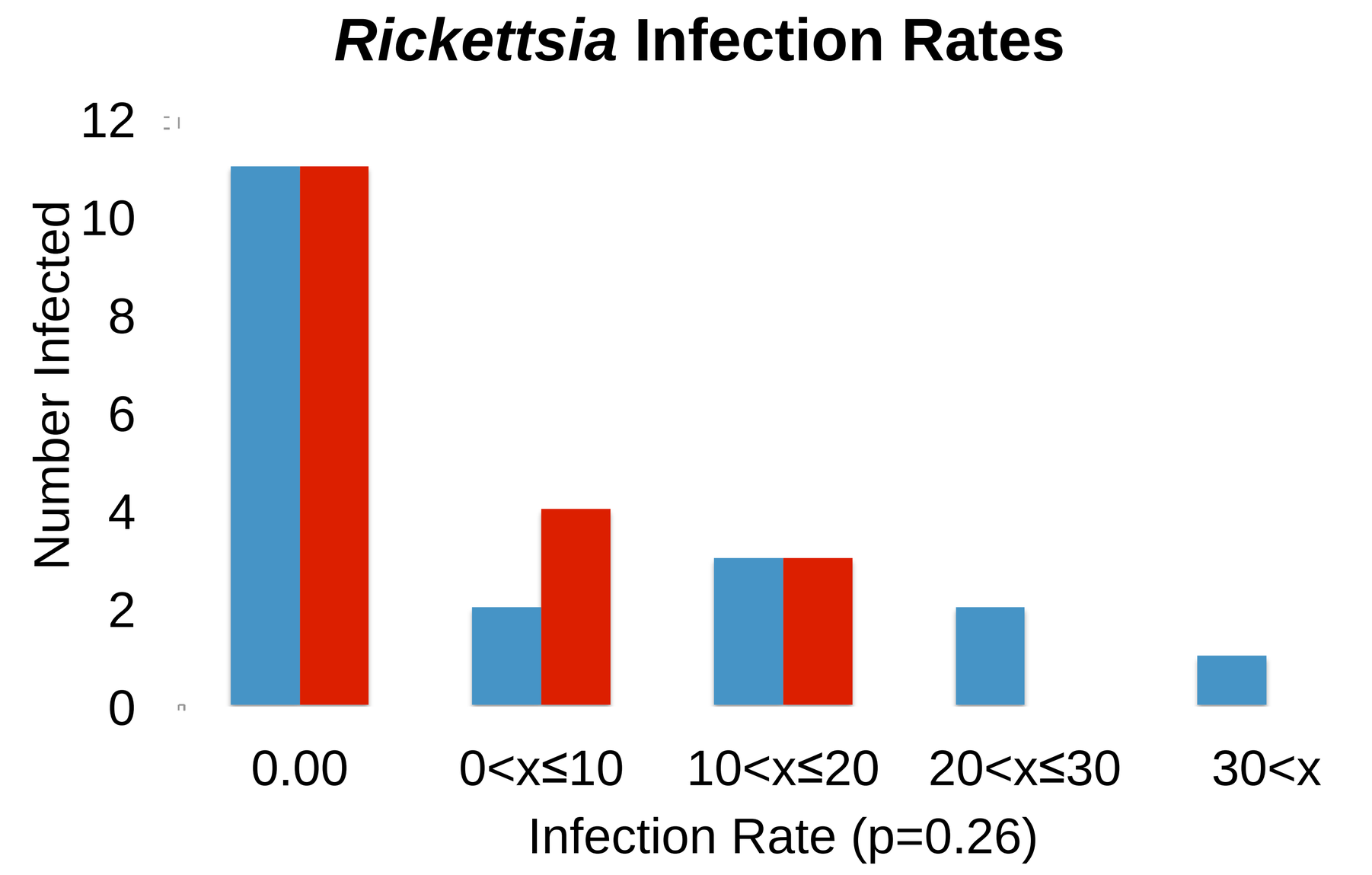


Legend
A-Robust Taq Neg. Control
B-2AAM2 with Robust Taq
C-2AAM3 with Robust Taq
D-HiFi Taq Neg. Control
E-2AAM2 with HiFi Taq
F-2AAM3 with HiFi Taq

Figure 5: Comparison of Robust and HiFi Taq Polymerase in First Round PCR

- Four types of pathogenic bacteria were found in Lone Star Ticks: *Coxiella*, *Rickettsia*, *Ehrlichia chafeensis*, and *Ehrlichia canis*.
- Robust and HiFi Taq Polymerase amplified comparable microbial communities (Figure 3 and 4).
- Males and females were found to have similar levels of *Rickettsia* infection.
- Female lone star ticks had higher *Coxiella* infection rates than males.





Further Directions

- Develop an assay to cheaply and easily screen ticks for human pathogens to determine if someone who was bitten is at risk.
- Look at the sequence variation among different pathogen types.

Acknowledgements

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References

[1] http://www.cdc.gov/ticks/maps/lone_star_tick.html
[2] https://www.flickr.com/photos/joshuallen/
[3] http://bugguide.net/node/view/51997
[4] http://entnemdept.ufl.edu/creatures/urban/medical/lone_star_tick.htm

Goddard J, Varela-Stokes AS. 2009. Role of the lone star tick, *Amblyomma americanum* (L.), in human and animal diseases. Vet Parasit 160:1-12.

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