Ticked Off:

Metagenomic Profiling of Tick RNA for Pathogenic Bacteria 16S Sequences

Luke Waldie (ID: 45287473)
Supervisor: Professor Stephen Barker (assisted by Samuel Kelava)

Introduction

Why examine ticks?

With the concerns around the spread of Lyme Disease in Australia (Collignon, Lum, & Robson, 2016) as well as other pathogenic bacteria carried by ticks (Dehhaghi et al., 2019), the ability to rapidly analysis large amounts of ticks for the presence of these disease inducing bacteria is needed. This was made apparent with the death of a patient with a undiagnosed *Rickettsia honei* infection (Graham et al., 2017). This project aimed at evaluating the viability of using pooled tick RNA to assess for the presence of bacteria via their 16S mitochondrial sequences.

Why RNA?

Whilst evaluation of host DNA for pathogen has been performed, such as Ravi et al. (2019) assessing the presence of Rickettsia bacteria in canines, the viability of this method of testing pathogenic species in ticks themselves has not been explored. Thus, this model was created in order to allow future epidemiological testing.

Why bacterial mitochondrial 16S?

Due to its presence in almost all bacteria, and its highly conserved nature, the ribosomal genes are an effective means of identifying bacterial species (Janda & Abbott, 2007). Due to its intrinsic role in protein synthesis, mutations do not often impact function and are an effective representation of evolution over time, allowing effective species identification (Yang et al., 2014).

Aim:

To construct and evaluate a metagenomic model for identifying pathogenic bacteria species from pooled tick RNA sequences obtained from Harvey et al. (2019)

Method

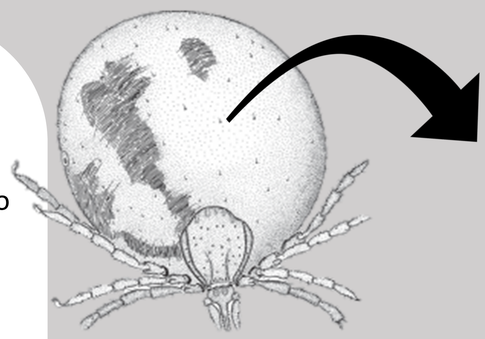
Harvey et al. (2019) utilised deep RNA sequencing to examine RNA viruses in Australian ticks) (refer to report for sequencing information). This data published on GenBank (PRJNA494273) has sufficient depth and sample size (n=11) to allow construction and testing of this model. This was downloaded, with Ixodes Holocyclus (n=9), Ixodes trichosuri (n=1) and Amplyomma moreliae (n=1) identified (4.9 ± 1.1 Gbase).

Data was imported to Geneious Prime (v.2021.1.1) alongside 12 pathogenic bacterial 16S sequences (Table 1; as defined by Dehhaghi et al. (2019)). 5 (red) were identified as known pathogens in Australia.

Each pool of RNA tick data was mapped to the 12 16S reference sequences (Fast Sensitivity). The produced mapped references were put through NCBI Blast+ (16S ribosomal RNA database, Discontinuous Megablast).

Table 1: Pathogenic bacteria with ticks as host (Dehhaghi et al.,2019) (Red are known **Australian** pathogens)

Name	Sequence Length
Francisella tularensis	1521
Burkholderia pseudomallei	1488
Bartonella henselae	1488
Borreliella burgdorferi	1477
Francisella hispaniensis	1474
Bartonella clarridgeiae	1466
Anaplasma phagocytophilum	1433
Coxiella burnetii	1465
Rickettsia honei	1491
Rickettsia australis	1415
Rickettsia marmionii	1388
Rickettsia gravesii	1293



Positive Hits

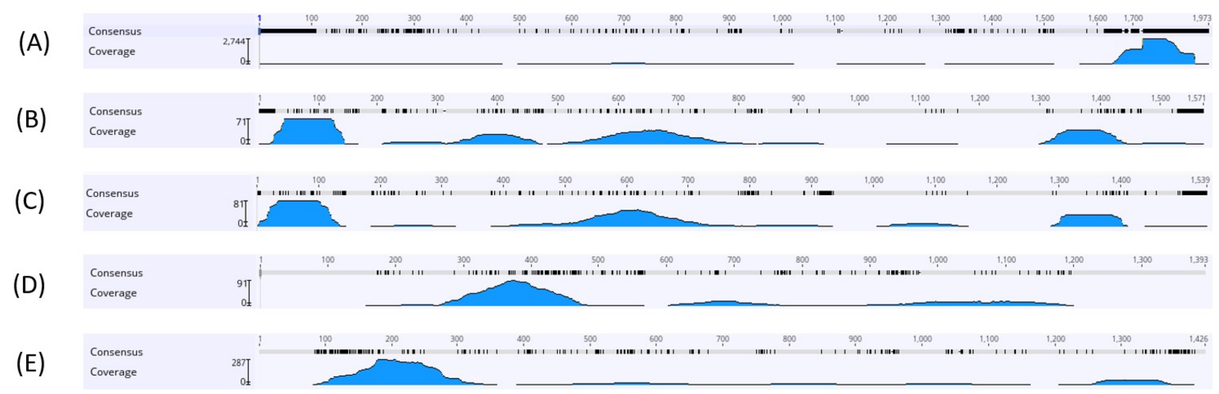


Figure 1: Map to Reference Consensus Sequences for *Rickettsia honei* and *Rickettsia australis* 16S for tick species that returned a positive NCBI Blast+. (A) SRR8054127 mapped to *Rickettsia honei* (B) SRR8054129 mapped to *Rickettsia honei*. (C) SRR8054131 mapped to *Rickettsia honei* (D) SRR8054131 mapped to *Rickettsia mamonii*. (E) SRR8054136 mapped to *Rickettsia australis*

Pathogenic Strains

Organism	Tick RNA Pool (Harvey et al., 2019)	Nucleotides Length	Identical Sites (%)	Position of Hit End	Position of Hit Start
Rickettsia honei	SRR8054127	1807	83.20	903	3
Rickettsia honei	SRR8054129	1814	82.50	907	1
Rickettsia honei	SRR8054131	2077	78.30	1208	171
Rickettsia honei	SRR8054131	2079	78.10	1222	183
Rickettsia australis	SRR8054136	2396	82.90	1365	167

Table 2: NCBI Blast+ positive returns for reference mapped 16S sequences

The NCBI Blast+ 16S bacterial sequence had 59 Rickettsia results, with 1 *Rickettsia hon*ei and 4 *Rickettsia australis*. Figure 2 demonstrates the reference mapped sequences, with Table 2 highlighting the positive return results.

Discussion

As seen in Table 2, five positive NCBI blast results were found in the species being examined. However, Figure 1 demonstrates the limited depth of the mapped sequences to their respective species. Tick RNA from SRR8054129 returned *R. honei* for both *R. honei and R. mamonii* mapped references, supporting their genetic homology (Unsworth et al., 2007).

Significance

The positive returns imply the viability of examining tick RNA pooled data as a means of assessing the presence of pathogenic bacteria. The positive pathogenic species supported epidemiological distribution of *Rickettsia* species (Unsworth et al., 2007; Stewart et al., 2017). Moreover, the modular nature of this model allows the testing for other pathogenic species in different host organisms. Future investigation may allow the implementation of RNA sequencing as a means of epidemiological identification.

Limitations

Conclusions from this model is limited due to the lack of validation.

No additional means of identification were obtained due to the base quantity of the RNA pooled data limiting other exploration methods (de novo assembly).

No published positive controls of RNA of confirmed infected tick. Whilst Ravi et al. (2019) explored *Rickettsia* in canines, this was as DNA and focused on Israeli strains rather than Australian.

Future testing with a known positive control is required to valid this method as clinically useful.

References

Barker, Stephen C, & Walker, Alan R. (2014). Ticks of Australia. The species that infest domestic animals and humans. Zootaxa, 3816(1), 1–144. https://doi.org/10.11646/zootaxa.3816.1.1

Collignon, P. J., Lum, G. D., & Robson, J. M. (2016). Does Lyme disease exist in Australia? *Med J Aust, 205*(9), 413-417. doi:10.5694/mja16.00824

Dehhaghi, M., Kazemi Shariat Panahi, H., Holmes, E. C., Hudson, B. J., Schloeffel, R., & Guillemin, G. J. (2019). Human Tick-Borne Diseases in Australia. *Frontiers in cellular and infection microbiology, 9*, 3-3. doi:10.3389/fcimb.2019.00003

Graham, R., Donohue, S., McMahon, J., & Jennison, A. V. (2017). Detection of Spotted Fever Group Rickettsia DNA by Deep Sequencing. Emerging infectious diseases, 23(11), 1911–1913. https://doi.org/10.3201/eid2311.170474

Harvey, E., Rose, K., Eden, J.-S., Lo, N., Abeyasuriya, T., Shi, M., . . . Holmes, E. C. (2019). Extensive Diversity of RNA Viruses in Australian Ticks. *Journal of Virology, 93*(3), e01358-01318. doi:10.1128/JVI.01358-18

Janda, J. M., & Abbott, S. L. (2007). 16S rRNA Gene Sequencing for Bacterial Identification in the Diagnostic Laboratory: Pluses, Perils, and Pitfalls. *Journal of Clinical Microbiology, 45*(9), 2761. doi:10.1128/JCM.01228-07

Ravi, A., Ereqat, S., Al-Jawabreh, A., Abdeen, Z., Abu Shamma, O., Hall, H., . . . Nasereddin, A. (2019). Metagenomic profiling of ticks: Identification of novel rickettsial genomes and detection of tick-borne canine parvovirus. *PLoS Negl Trop Dis, 13*(1), e0006805. doi:10.1371/journal.pntd.0006805

Stewart, A., Armstrong, M., Graves, S., & Hajkowicz, K. (2017). Rickettsia australis and Queensland Tick Typhus: A Rickettsial Spotted Fever Group Infection in Australia. The American journal of tropical medicine and hygiene, 97(1), 24–29. https://doi.org/10.4269/ajtmh.16-0915

Unsworth, N. B., Stenos, J., Graves, S. R., Faa, A. G., Cox, G. E., Dyer, J. R., Boutlis, C. S., Lane, A. M., Shaw, M. D., Robson, J., & Nissen, M. D. (2007). Flinders Island spotted fever rickettsioses caused by "marmionii" strain of Rickettsia honei, Eastern Australia. Emerging infectious diseases, 13(4), 566–573. https://doi.org/10.3201/eid1304.050087

Yang, L., Tan, Z., Wang, D., Xue, L., Guan, M.-X., Huang, T., & Li, R. (2014). Species identification through mitochondrial rRNA genetic analysis. *Scientific reports, 4*, 4089-4089. doi:10.1038/srep04089