From microbiomes to road kill: What DNA can tell us about the ecology of ticks

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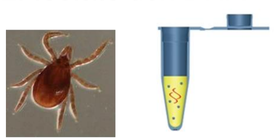
ABSTRACT

Knowledge of host species that maintain populations of *Ixodes scapularis*, the vector of Lyme disease in the eastern U.S., is needed to improve preventative strategies. The prevailing dogma of Lyme disease ecology is that the white-footed mouse (*Peromyscus leucopus*) is the most important host for juvenile ticks (i.e., larvae and nymphs; 1). The white-tailed deer (*Odocoileus virginanus*) is the primary host for adult ticks, however its role as a host for juveniles has not been rigorously studied. Here we provide both DNA and visual evidence that juveniles feed on *O. virginanus.* Our DNA-based host detection system may be more broadly applied in future studies to identify other important hosts of *I. scapularis*.

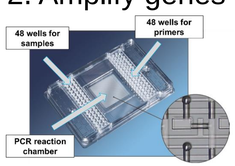
METHODS

1. DNA was extracted from nymphal- and adult- stage *I. scapularis* (N= 105 and 38, respectively; see below for the workflow) in southern Vermont, USA.
2. Genes of interest were amplified using parallel PCR on Fluidigm Access Array plates and four primer sets (Table 1).
3. Amplicons were sequenced on an Illumina HiSeq machine.
4. Bioinformatics was performed with QIIME2 (2; Quantitative Insights Into Microbial Ecology) software.

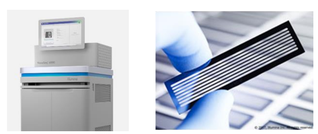
1. Isolate tick DNA



2. Amplify genes



3. DNA sequencing



4. Bioinformatics

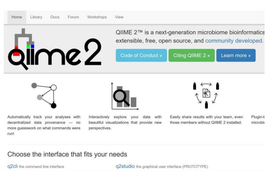


Table 1. Primer sets used in parallel PCR

Primer set	Gene region	Target group	Amplicon Size	Reference
566F/1200R	18S	Protists	>660	3
12S-F/9R	12S	Vertebrates	145	4
18S-35F/49R	18S	Vertebrates	115-210	5
16Scp-F/16Scp	16S	Vertebrates	294	6

RESULTS

We detected *Babesia odocoilei*, the agent of cervid Babesiosis in white-tailed deer (Fig. 1), in nymphal stage ticks. This suggests feeding on deer in the larval stage. We detected *O. virginianus* DNA in 100% of adult ticks, suggesting feeding on deer during the larval and/or nymphal stage (Table 2).

Eukaryotic microbiome

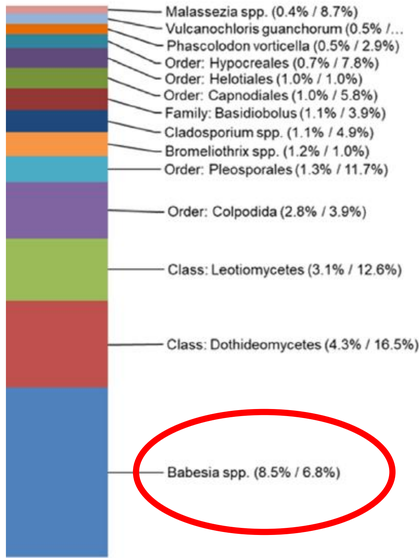


Figure 1. Taxonomy summary for the 566F/1200R primer set. Parentheses: sequences as pct of total/pct of samples.

Table 2. DNA-based host detection

Primer set	Top ASV	Samples	Classification	Percent Identity: Custom	Probe	NCBI	E score
12S-6F/9R	[1]ASV1	34/38	*O. virginianus*	100%	100%	100%	2 E-32
18S-35F/49R	[2]ASV3	38/38	*O. virginianus*	100%	100%	100%	1 E-46
16Scp-F/16Scp	[3]ASV1	27/38	*O. virginianus*	100%	NA	100%	5 E-150

- We inspected hides from road-killed deer for direct visual evidence of juvenile *I. scapularis* feeding.
- We found 38 larval-stage ticks on one deer (Fig. 2 left panel).
- Direct inspection of a second deer revealed six embedded nymphs (Figs 2a – c).

Visual confirmation of DNA analyses

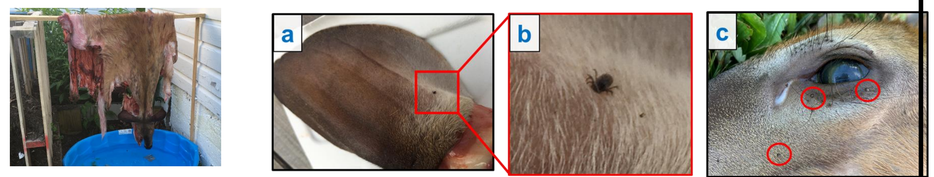


Figure 2. Larval ticks were collected from a road-killed deer by hanging a hide over a pool of water (left). Nymphs were found on the ear and near eyes of a 2nd deer (a-c)

DISCUSSION

- Multiple lines of evidence indicate that juvenile *I. scapularis* feeding on white-tailed deer is more common than previously believed.
- Knowledge of juvenile feeding behavior will inform new management strategies for tick control by targeting these life stages on deer.
- Our DNA-based host detection system may be more broadly applied to improve understanding of tick feeding behavior in future studies.

REFERENCES

[1]LoGiudice, K., et PNAS, 2003. 100(2): p. 567-71; [2]Bolyen E, et al. PeerJ Preprints, 2018; [3]Hadziavdic K, et al. PLoS One 2014;9:e87624; [4]Goessling, L.S., et al., Journal of Medical Entomology, 2012. 49(3): p. 772-776; [5]Allan, B.F., et al., Emerging Infectious Diseases, 2010. 16(3): p. 433-40; [6]Tessler, M., et al., Systematics and Biodiversity, 2018. 16(5): p. 488-496; [7]Landesman, William J., et al. FEMS Microbiology Ecology 95.12 (2019): fiz167

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