Title: Nanopore Sequencing Enables Broad Detection and Surveillance of Tick-Borne Pathogens in *Ixodes scapularis*

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HIGHLIGHTS

- ONT workflow enables unbiased metagenomic detection of tick-borne pathogens
- Approach achieves high precision and sensitivity across major pathogen groups
- Workflow supports simultaneous detection of bacterial, protozoal, and viral taxa
- Quantitative validation confirms dose-responsive detection of pathogen DNA

IN BRIEF

We present a metagenomic sequencing workflow for detecting tick-borne pathogens using Oxford Nanopore long-read technology. Direct sequencing of *Ixodes scapularis* genomic DNA enables unbiased identification of bacterial, protozoal, and viral taxa. Quantitative validation demonstrates its potential for real-time genomic surveillance and future strain-level resolution.

SUMMARY

Surveillance of tick-borne diseases in the United States largely relies on PCR-based detection of predefined targets, limiting the ability to identify emerging pathogens or characterize tick-associated microbiomes. The blacklegged tick (*Ixodes scapularis*), the primary vector of several medically important pathogens including *Borrelia burgdorferi*, *Babesia microti*, and *Anaplasma phagocytophilum*, is of particular relevance in the eastern United States. To address limitations of conventional testing, we developed an Oxford Nanopore Technologies (ONT) metagenomic sequencing workflow incorporating adaptive sampling for real-time, field-deployable detection. We sequenced genomic DNA from *I. scapularis* ticks provided by the Connecticut Agricultural Experiment Station Tick Testing Laboratory (CAES-TTL) and performed taxonomic classification using Kraken2, enabling simultaneous detection of bacterial, protozoal, and viral taxa. Our threshold-based pipeline demonstrated high precision (0.902) and sensitivity (0.668). Validation with cultured *B. burgdorferi* DNA (0–200 fmol) showed a dose-dependent relationship ($R^2 = 0.62$), confirming quantitative responsiveness and supporting future strain-level genomic surveillance.