Can the Gram-negative bacterium *Escherichia coli* colonize the gut of Lone Star Tick (*Amblyomma americanum)*?

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Abstract

Ticks are obligate blood feeding ectoparasites and vectors of several mammalian pathogens (Williams-Newkirk et al, 2014). In addition to pathogens they also carry a bacterial community with commensal and symbiotic relationships (Bonnet et al, 2017). Using a culture-dependent approach we previously reported a high prevalence of Gram-positive bacteria in the gut of field collected lone star ticks (*Amblyomma americanum*). These results suggested that epithelial immunity functions to control Gram-negative bacteria in *A. americanum.* In this study, we used a culturing and non-culturing approach to measure the outcome of *E.coli* (Gram-negative) when fed to female adult lone star ticks (n=16). Results showed a significant reduction of *E.coli* at Days 1, 3 and 7 post bacterial feeding. qPCR of 16S rDNA confirmed reduction of bacterial rDNA when compared to water fed ticks (n=16). Our results suggest that there is a midgut epithelial immune response in place, which mainly targets Gram-negative bacteria

Purpose

To asses the outcome (potential colonization) of Gram-negative bacterium (*E. coli*) in the gut of the lone star tick; when introduced by capillary/forced feeding to laboratory-reared unfed adult female *A. americanum.*

Hypothesis: Gram-negative bacteria are not able to colonize the midgut of the lone star tick (*A. americanum*) and are not deleterious to the tick when introduced by capillary feeding in high concentrations (≈1,000 CFU/μL).

Study System

The lone star tick is a common species in the Midwest and eastern United States (Figure 1) and a known vector of pathogens that cause disease to mammals (e.g. Ehrlichiosis, Tularemia, lyme-like and others) (Sayler et al, 2016, CDC 2016). It is considered a very aggressive tick that bites humans; most frequently from early spring to late fall. The adult female is distinguished by a white dot or "lone star" on her back (CDC 2016).

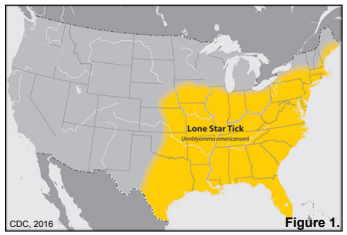


Figure 1.

Methods and Experimental Design

Female *A. americanum* were obtained by the Oklahoma State Tick Rearing Facility (n=32). Ticks were preconditioned for 24 hrs (30%R.H and 26˚C) before feeding and placed on dental wax. 454 µm microcapillary tubes were introduced into hypostomes containing the *E. coli* inoculum. Ticks were allowed to drink for 1 hr and controls drank water voluntarily through a 5 μL droplet *ad libitum* Images 1-3).

Bacterial inoculum was prepared using sterile deionized water until turbidity reached O.D. at 0.200. Ticks were fed the 100x diluted inoculum (1,032 CFU/μL) and bacterial ingested amount was measured through capillary tube diameter (1mm=0.1464 μL).

Methods and Experimental Design

After feeding; 4 ticks were selected for processing (2 for plating on bacterial media and 2 for qPCR). Same number of ticks were selected from controls (water fed). Tick sampling was conducted at days 0, 1, 3 and 7 Post-Feeding (D0PF, D1PF, D3PF and D7PF). Before processing ticks were surface sterilized with 0.5% sodium hypochlorite, 70% ethanol, and washed with sterile water. Individual ticks were processed by dissection on sterile wax surface and extraction of soft tissues (midguts included). Soft tissues were homogenized in phosphate buffer saline, spread plated on trypticase soy agar, and incubated at 37˚C for 24 hrs (Images 4-7). Homogenates for qPCR were stored at -80˚C until needed.

DNA extraction for 16S qPCR was done using a ZymoBiomics DNA miniprep kit and DNA concentration was measured using Nanodrop 2000 spectrophotometer. Amplification by qPCR of 16S was conducted using universal eubacterial primers for 16S rDNA. V-ATPase qPCR (tick single copy gene) was used as reference for 16S amount calculation. 16S DNA changes were estimated using the delta Ct method.

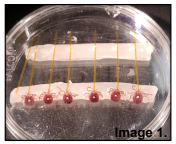
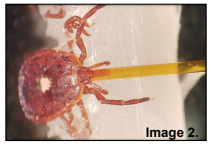


Image 1.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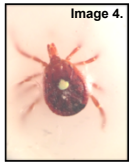
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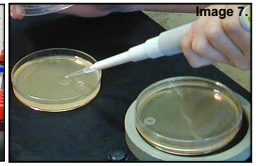
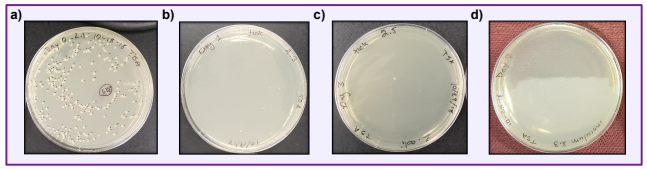


Image 7.

Results

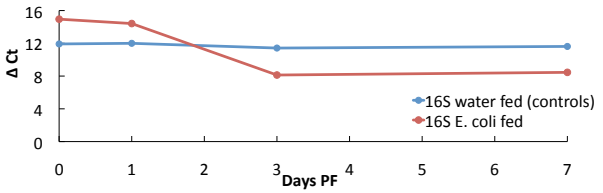
Ticks fed with *E.coli* (n=16) drank an average of 502 cells/tick during 1 hr. A decrease in cell numbers was observed at D0PF (30 min PF) with an average of 195 CFU/tick. We observed a dramatic decrease in *E. Coli* CFU, from D0PF (195 CFU) to D1PF (0 CFU). This decrease was maintained over sampling times (D3PF=1 CFU and D7PF=0 CFU). Water fed controls presented 0 CFU/tick at all time points (Figure 2).

Figure 2. E.coli recovered from the gut of *Amblyomma americanum* at D0PF, D1PF, D3PF and D7PF. a) D0PF (195 CFU/tick), b) and d) D1PF and D7PF(0 CFU/tick) and c) D3PF (1 CFU/tick).



Delta Ct of 16S rDNA qPCR and V-ATPase (reference gene); showed decrease of total 16S rDNA amount, which suggest the reduction of *E.coli* (Figure3).

Figure 3. Delta Ct of 16S rDNA in *E.coli* fed and water fed *A. americanum* over time



Discussion and Conclusions

Using a culture-dependent approach we previously reported that the midgut bacterial community of the lone star tick is heavy biased towards Gram-positive taxa (89%). These results indicate that epithelial immunity functions to control Gram-negative bacteria in *A. americanum.*

In this study we assessed the outcome of a Gram-negative bacterium (*E. coli*) when fed to adult non-fed laboratory reared female lone star ticks. With this; we aim to indirectly have a better understanding of the tick midgut immunity and microbial homeostasis of *A. americanum.* Our results show that even high bacterial loads of a Gram-negative bacterium can be cleared out from the midguts of lone star ticks within less than 24 hrs. Our findings could potentially be used to assess the importance of extracellular bacterial communities in tick fitness and gut microbial homeostasis by conducting co-infection experiments; i.e. if they can prevent or allow the establishment of tick-borne pathogens as suggested in other ixodid ticks (Narasimhan et al., 2014 and Abraham et al., 2017).

Future Directions

Based on our previous findings of a heavy biased midgut bacterial community towards Gram-positive bacteria; we plan to asses if Gram-positive bacteria, isolated from field collected ticks, are able to colonize the midgut of the lone star tick in the laboratory setting. In addition; we want to replicate this experiment to verify that our results are consistent, using *E. coli* and other Gram-negative bacteria and conduct competition/co-infections experiments. For this; we will use the representative bacterial isolates obtained from the field ticks, to assess the tick midgut colonization and homeostasis as well as to study the tick midgut epithelial immunity.

References

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