

Laboratory colonization by *Dirofilaria immitis* alters the microbiome of female *Aedes aegypti* mosquitoes

Abdulsalam Adegoke

University of Southern Mississippi

Erik Neff

University System of Georgia

Amie Geary

University of Southern Mississippi

Montana Ciara Husser

University of Southern Mississippi

Kevin Wilson

University of Southern Mississippi

Shawn Michael Norris

University of Southern Mississippi

Guha Dharmarajan

University System of Georgia

Shahid Karim (✉ Shahid.Karim@usm.edu)

The University of Southern Mississippi <https://orcid.org/0000-0001-5596-3304>

Research

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Abstract

Background The ability of blood feeding arthropods to successfully acquire and transmit pathogens of medical and veterinary importance has been shown to be interfered or enhanced by the arthropod's native microbiome. Mosquitoes transmit bacteria, viruses, protozoan and filarial nematodes, majority of which contributes to the 17% of infectious disease cases worldwide. *Dirofilaria immitis*, a mosquito transmitted filarial nematodes of dogs and cats, is vectored by several mosquito species including *Aedes aegypti*.

Methods In this study, we investigated the impact of *D. immitis* colonization on the microbiome of laboratory reared female *A. aegypti*. Metagenomic analysis of the V3-V4 variable region of the microbial 16S rRNA was used for identification of the microbial differences down to species level.

Results We generated a total of 1068 OTUs representing 16 phyla, 181 genera and 271 bacterial species. Overall, in order of abundance, Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes were the most represented phylum with *D. immitis* infected mosquitoes having more of Proteobacteria (71%) than uninfected mosquitoes (56.9%). An interesting finding in this study is the detection of *Klebsiella oxytoca* in relatively similar abundance in infected and uninfected mosquitoes. It has been previously shown to indirectly compete for nutrients with fungi on the domestic housefly eggs and larva. While not statistically significant, *D. immitis* infection alters bacterial diversity by reducing the bacterial species richness and abundance.

Conclusions In conclusion, this is the first study to the best of our knowledge to understand the impact of a filarial infection on the microbiome of its mosquito vector. While the microbiome composition of *A. aegypti* mosquito have been previously reported, our study shows that in an effort to establish itself, a filarial nematode modifies and alters the overall microbial diversity within its mosquito host.

Background

Dirofilaria immitis, responsible for canine respiratory and associated heartworm disease, is a filarial nematode that mainly infects wild and domestic canids, such as dogs and coyotes (Bowman and Atkins, 2009). While primarily a parasite of veterinary importance, human infection has also been reported, albeit rarely (Theis, 2005). With over a 100,000 dogs affected annually, *D. immitis* has been identified as the most important parasite affecting dogs in the United States (Bowman et al., 2016). Beyond the United States, canine dirofilariasis also poses significant problems in dogs in Australia, Europe and Asia (Morchon et al., 2012).

Over 70 species of mosquitoes have been reported to transmit *D. immitis* (Wang et al., 2014), and 28 of these are found in the United States (Ludlam et al., 1970). The epidemiology and maintenance of *D. immitis* have been made significant by the abundance of the mosquito vectors. While canine infection with *D. immitis* is endemic in the United States, seroprevalence studies have shown that prevalence amongst domestic dogs is highest in the Southeastern states (Little et al., 2014). The United States Southeast have also shown to have an increased incidence of canine heartworm disease when compared

to other states (Drake and Wiseman, 2018). A major reason for this could be because about half of the mosquito species known to transmit *D. immitis* are found in the Southeastern United States (Lok et al., 2000). Prevention and control of canine heartworm disease has primarily focused on the use of therapeutics such as ivermectin and other macrocyclic lactones. While these therapeutics have proven to be effective in killing and preventing establishment of third and early fourth-stage larva (Bourguinat et al., 2015), recent studies have shown significant resistance against these drugs of choice (Bourguinat et al., 2011, Geary et al., 2011).

In addition to being an important vector of human and animal pathogens, mosquitoes and other arthropod vectors also host highly complex and diverse microbial communities. Members of these microbial communities have obligate relationships with mosquitoes, and are maintained vertically through mosquito generations (Guégan et al., 2018) or acquired from the environment (Engel and Moran, 2013; Strand 2017). The microbiome of mosquitoes have been shown to help maintain normal midgut functions, as well as interfering or aiding vector competence of the mosquito (Strand, 2018; Jayme et al., 2019). As previously shown, pathogen susceptibility and vector competence of an arthropod can be modulated by the microbial composition within the arthropod vector (Bonnet et al., 2017).

Female mosquitoes are competent vectors for a variety of viruses, protozoan and filarial nematodes of public health significance (Bandyopadhyay et al., 2006). Of significant veterinary importance in North American countries and some other parts of Europe is *Dirofilaria immitis*, a filarial nematode that causes canine heartworm disease (Ledesma et al., 2019). Canine heartworm disease is a serious life-threatening disease of canines and other domestic pets in which adult worm lodges in the cardiopulmonary circulation of infected dogs causing cardiac necrosis which leads to ischemic hypoxia and a total shutdown of the respiratory and cardiovascular system (Spence Beaulieu et al., 2019). A significant number of mosquito species have been reported to vector the infective stage of the parasite (Ledesma et al., 2019), one of which is *A. aegypti* which is one of the most predominant temperate mosquitoes (Ledesma and Harrington, 2011).

Aedes aegypti is an important vector responsible for the transmission of many pathogens of humans (e.g., Zika, Dengue, Chikungunya Viruses; Alto et al., 2008; Marchette et al., 1969; Martin et al., 2010) and animals (e.g., *D. immitis*; Erickson et al. 2009). *Dirofilaria immitis* is a parasite that causes severe and potentially fatal infections to dogs worldwide (Bowman and Atkins 2009). Adult female *D. immitis* parasites release microfilariae (mf) into the blood stream of the vertebrate host, usually a dog, which are subsequently taken up by adult female mosquitoes during their blood meal (Bowman and Atkins 2009, Evans 2011). Microfilaria in the blood enter the midgut of the mosquito during feeding and subsequently migrate to the Malpighian tubules. In the Malpighian tubules of the mosquito mf develop into first stage larvae and molt twice to reach their infective L3 stage, which migrate to the head and proboscis of the mosquito and can infect the next dog during the next blood meal (Bowman and Atkins 2009, Evans 2011). The time it takes for the parasite to develop, known as the extrinsic incubation period (EIP), averages 14 days within this mosquito (McCall 1981).

Previous studies have demonstrated significant success in reducing vector competence of mosquitoes for pathogenic microbes by taking advantages of the mosquito microbiome and pathogen interactions (Ramirez et al., 2012; Dennison et al., 2014). It is an established fact that pathogens must compete with the native microbial community in their vector host in order to propagate and facilitate subsequent transmission to a susceptible host (de la Fuente et al., 2017; Bonnet et al., 2017). Hence, understanding the pathogen-microbiome interaction in vectors of significant public health importance is important. Our study will contribute to existing knowledge on controlling vector transmitted pathogens.

Therefore, the objective of this study was to test the hypothesis that differences will exist in the microbiome composition of a mosquito species (*Aedes aegypti*) that is infected with a filarial pathogen (*Dirofilaria immitis*) when compared to uninfected mosquitoes from the same laboratory colony. The goal of this study was to establish specific synergistic or antagonistic interactions between the *D. immitis* and members of the microbial communities within the mosquito vector. This we believe will further add to existing knowledge on how arthropod microbial communities affects vector competence and will contribute to taking a step towards controlling arthropod-transmitted pathogens of public health significance.

Methods

Study organisms

The mosquito used for investigation was the *A. aegypti*, Liverpool Blackeye strain, a highly susceptible mosquito strain used predominately in research for *Aedes* sp. (Buxton & Mullen 1981).

Mosquito maintenance: *Aedes aegypti*, originally obtained from the Filariasis Research Reagent Resource Center (FR3) (Michalaski et al. 2011), were raised under standard laboratory conditions – 27 °C, 80 + 5% relative humidity, and a 12:12-hour light diurnal cycle (Dharmarajan et al. 2019). Adult female mosquitoes were blood fed using artificial membrane feeder. One day prior to membrane feeding 50 female mosquitoes were transferred to ~ 500 mL plastic containers with mesh tops (henceforth “cages”).

Females were starved of sugar for 12 hours and deprived of water for four hours prior to blood feeding. Mosquitoes in each cage were allowed to feed for two hours or until repletion on a Parafilm membrane stretched over an inverted water-jacketed glass membrane feeder maintained at 40°C. Each feeder was filled with 200 uL of dog blood infected or uninfected with *D. immitis*. The dog blood was obtained from FR3.

Dirofilaria immitis detection

Prior to screening for *D. immitis* infection from mosquitoes, DNA from individual mosquito samples was extracted using a commercially available kit (QIAGEN, DNeasy Blood & Tissue Kit) and quality was confirmed using a nanodrop machine. All 62 mosquito samples were screened for *D. immitis* infection irrespective of whether they were fed on infected or uninfected blood by amplifying the COI gene (656 bp) of the *D. immitis* mitochondrial DNA (Murata et al., 2003). Briefly, a 25 µl reaction was set up comprising 1 µl each of the COI-Forward (5′–TGATTGGTGGTTTTGGTAA–3′) and COI-Reverse (5′–

ATAAGTACGAGTATCAATATC–3') primers, 12.5 µl of 2X mastermix (New England Biolabs), 2.5 µl DNA template and 8.5 µl of nuclease free water. For each cycle that was run, a *D. immitis* infected blood sample and nuclease free water were simultaneously included as positive and negative controls respectively. The PCR cycle comprises of an initial denaturation step at 94°C for 5 minutes and 40 cycles of 94°C for 1 minute, annealing at 50°C for 2 minutes and extension at 72°C for 3 minutes. A final extension at 72°C for 5 minutes and an infinite hold at 4°C.

Confirmation of amplification was done by loading the PCR products in a SYBR safe stained gel. Briefly, 2% gel was made by autoclaving a solution of 1X TAE buffer and molecular grade agar. SYBR safe stain (1 µl SYBR safe: 10 mL TAE buffer) was added to the agar solution, poured into a precast gel tray and allowed to cool. To load the samples onto the gel, 6 µl of PCR product was mixed with 4 µl of 6X dye and pipetted into the wells. Lastly, 5 µl of a low molecular weight DNA ladder was loaded onto the gel and the gel could run for 45 minutes at 100 V. Amplified PCR products were viewed using a Chemidoc gel imager.

16S rRNA library preparation and sequencing

Six individual mosquito genomic DNA were pooled to make one biological replicate and five biological replicates each of *D. immitis* infected and uninfected pools were prepared for metagenomic analysis. PCR sequencing of the V1-V3 variable region of the bacterial 16S rRNA gene were amplified using barcoded primers 27F/519R as outlined by the 16S illumina's MiSeq protocol (www.mrdnalab.com, Shallowater, TX, USA). Briefly, PCR was performed using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94 °C for 3 minutes, followed by 30–35 cycles of 94 °C for 30 seconds, 53 °C for 40 seconds and 72 °C for 1 minute, after which a final elongation step at 72 °C for 5 minutes was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Then the pooled and purified PCR product were used to prepare Illumina DNA library. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq following the manufacturer's guidelines.

Microbiome analysis

Sequence analysis was done using the Quantitative Insights into Microbial Ecology (QIIME 2), unless stated otherwise. Briefly, processing of raw fastq files were demultiplexed. The Atacama soil microbiome pipeline was incorporated for quality control of demultiplexed paired-end reads using the DADA2 plugin as previously described. Sequence alignment and subsequent construction of phylogenetic tree from representative sequences was done using the MAFFT v7 and FastTree v2.1 plugin. Operational taxonomic assignment was done using the qiime2 feature-classifier plugin v7.0 which was previously trained against the SILVA 132 database preclustered at 99%. Raw data from this analysis were submitted deposited and assigned the GenBank BioProject number # PRJNA606536.

Results

***immitis* infection alters relative abundance of bacteria taxa in *A. aegypti*.** Taxonomic assignment was done against the SILVA database to observe for the difference in microbiome composition and relative abundance of bacteria species. Both infected and uninfected mosquitoes possess similar composition of bacteria taxa with differences observed in the relative abundances of specific bacteria species. The phylum Proteobacteria and Bacteroidetes were present in higher abundance in both mosquito groups. Mosquitoes infected with *D. immitis* had relatively higher abundance of Proteobacteria (71%) with lower amount of Bacteroidetes (27%), while uninfected mosquitoes have lower amount of Proteobacteria (56.9%) and a higher abundance of Bacteroidetes (37%) (Fig. 1). Among the detected bacteria genera, the genus *Klebsiella* were detected at relatively similar abundances in both infected (36.3%) and uninfected (34.6%) mosquitoes (Fig 2). The genera *Ochrobactrum*, *Sphingobacterium*, and *Pseudomonas* were present exclusively in uninfected mosquitoes albeit at an abundance of 4.4%, 3.6% and 3% respectively (Fig 2). The genera *Enterobacter* (26.1%) and *Elizabethkingia* (9.4%) were in higher abundance in *D. immitis* infected mosquitoes (Fig 2). Except for *Klebsiella oxytoca*, which was present in similar abundance for infected (36%) and uninfected (34%) mosquitoes (Fig. 3). *Enterobacter hormaechei* (24.4%) and *Elizabethkingia meningoseptica* (9.4%) were more abundant in infected mosquitoes, while *Chryseobacterium indolgenes* (27.4%), *Grimontella senegalensis* (3.9%), and *Ochrobactrum spp* (4.4%) were more abundant in uninfected mosquitoes (Fig.3).

***immitis* infection affects species diversity in *A. aegypti*.** Following demultiplexing of paired end reads and quality control by removing chimera, we normalized OTU counts for individual biological replicates and a rarefaction curve was generated at a depth of 2000 (Fig. 4A). Adequate depth coverage was reached as evidenced by the individual curves plateauing out on the rarefaction curve. Our results indicated that phylogenetic diversity, estimated using Faith's phylogenetic distance and number of observed OTUs, was reduced in infected mosquitoes compared against uninfected mosquitoes (Fig. 4B and C). We also visualized the similarities and differences in the microbial composition of infected and uninfected mosquitoes by carrying out Principal Components Analysis on the Bray Curtis and the unweighted UniFrac distance matrixes (Fig. 4D and E). Figures 4D and E shows distinct clustering of *D. immitis* infected mosquito replicates with no outliers.

Discussion

To the best of our understanding, this study is the first to utilize 16S metagenomic analysis to understand the significance of a filarial nematode on the microbiome of a mosquito vector under laboratory conditions. This technique offers the advantage of detecting both culturable and unculturable pathogenic and non-pathogenic microbes from a given DNA or RNA. As previously reported from previous metagenome studies on mosquitoes and similar arthropods or insects (Mancini *et al.*, 2018; Coon *et al.*, 2014; Coatsworth *et al.*, 2018), Figure 1A shows that the phylum Proteobacteria, Bacteroidetes,

Actinobacteria and Firmicutes was detected from the mosquito groups tested. Similar studies have also identified the phylum Proteobacteria as one of the dominant phylum in the microbiome of *A. aegypti* mosquitoes (Ramirez *et al.*, 2012; Audsley *et al.*, 2017). We observed an inverse relationship in the abundances of the phylum Proteobacteria and Bacteroidetes in our study with *D. immitis* infected mosquitoes having a higher abundance of the phylum Proteobacteria when compared to the uninfected mosquitoes and vice-versa. The Proteobacteria group are the largest phylum found in different environments, plants and animals (<http://www.bacterio.net/>; Degli and Martinez, 2017) with members ranging from free living commensals to pathogenic microbes. We found that the genus *Klebsiella* was present in relatively similar abundances in both infected and uninfected mosquitoes. Although no known function has been associated to this group in insects or arthropods, *Klebsiella* belongs to a class of gram Proteobacteria known as Gammaproteobacteria with previous detection in field and laboratory raised *Culex quinquefasciatus*, *A. albopictus*, and *A. aegypti* (Gonçalves *et al.*, 2019; Yadav *et al.*, 2015; Wu *et al.*, 2019).

The presence of the genus *Enterobacter* in infected mosquito at approximately 20 folds of uninfected mosquitoes was also observed. This bacteria genus has been detected in similar microbiome studies of *A. aegypti* where they have been associated with their role in blood meal digestion due to their hemolytic activities (Coon *et al.*, 2016). This most likely explains why the genus *Enterobacter* have been commonly associated with females of other mosquito species (Minard *et al.*, 2013). A likely explanation for the increase in the abundance of this genus in the presence of *D. immitis* colonization could be a response to deplete the abundance of the filarial nematode through specific mechanisms by the bacteria. An elegant study by Cirimotich and colleagues showed the inhibition of *Plasmodium* infection in *Anopheles gambiae* mosquitoes mediated by a bacterium designated as *Enterobacter* Esp_Z. The inhibition was due to the production of reactive oxygen species by the bacteria. The presence of *Enterobacter* could also facilitate or enhance mosquito infection by *D. immitis*. Bacteria have also been shown to facilitate pathogen infectivity within arthropod vectors. One of such ways they achieve this is by producing enzyme-like substances that degrades compartment barriers within the arthropods which then aids in efficient colonization by the pathogen. Enhancins or enhancing-like proteins are produced by baculovirus such as *Tricoplusia ni* granulovirus and bacteria such as *Bacillus* and have been shown to degrade the peritrophic matrix of insect hence facilitating gut colonization by pathogens (Toprak *et al.*, 2012; Fang *et al.*, 2009; Parkhill *et al.*, 2001). A recent study on the enhancing producing commensal bacteria from *A. aegypti* identified *Enterobacter* as an enhancin expressing bacteria (Wu *et al.*, 2019).

Another bacteria genus that also increased with the presence of *D. immitis* infection is *Elizabethkingia*. Previous report has identified this genus from the midgut of laboratory reared (Gonçalves *et al.*, 2019) and field collected (Audsley *et al.*, 2017) *A. aegypti*. These studies did not associate any known role to this bacterium.

Bacteria in the genus *Chryseobacterium*, *Ochrobactrum*, *Sphingobacterium*, and *Pseudomonas* were all present in higher abundance in uninfected mosquito group. The detection of *Chryseobacterium* from our study agrees with similar detection from previous report in laboratory reared mosquitoes (Chen *et al.*, 2016; Wang *et al.*, 2011). *Pseudomonas*, a Gram-negative, Gammaproteobacteria was previously shown to have reduced abundance in *Wolbachia* positive *A. aegypti* mosquitoes. A similar observation was also made from our study where the abundance of *Pseudomonas* was inversely correlated with the presence of *D. immitis* infection in the mosquito as shown in Figure 2.

Bahia *et al.* reported blocking of the *Plasmodium* parasite by *Pseudomonas putida* isolated from *A. gambiae*.

Unexpectedly, only few bacteria species were differentially altered in *D. immitis* infected and uninfected mosquitoes. *Klebsiella (K.) oxytoca*, a Gammaproteobacteria belonging to the genus *Klebsiella*, was found at relatively similar abundance in infected and uninfected mosquitoes (Fig 3). A possible explanation for the presence of *K. oxytoca* in similar abundances in infected and uninfected mosquitoes could be an indication of the bacteria been a likely endosymbiont that could be vertically and horizontally acquired as previous studies have reported the detection of *K. oxytoca* from laboratory reared and field collected *C. quinquefasciatus*, *A. aegypti* (Gonçalves *et al.*, 2019). A symbiotic role will explain the reason why it was detected in such abundances in both mosquito groups. A probiotic role has also been associated with the bacteria in male medfly (*Ceratitis capitata*), where it was shown to reverse radiation induced loss of copulatory performance (Ami *et al.*, 2009). A study on the fungicidal effects of bacterial colonies found on the domestic housefly eggs revealed that fungal growth is inhibited by the presence of *K. oxytoca* by producing antifungal metabolites and nutrient depletion (Lam *et al.*, 2009). Determining the microbial communities in mosquito eggs and their antimicrobial activities will give a broader insight into members that inhibit microbial growth. The expression of Enhancin proteins by certain bacteria species have been documented and shown to breakdown the peritrophic matrix of insect vectors (Fang *et al.*, 2009; Parkhill *et al.*, 2001). Wu *et al.* (2019) recently showed that *Serratia (S.) marcescens* facilitates dengue virus invasion of *A. aegypti* by producing the protein Enhancin which breaks down the mucin component of the peritrophic matrix barrier. Interestingly, the same study also reported that *K. oxytoca* expresses an Enhancin protein that degrades another type of mucin in *A. aegypti* but with no effect on dengue infection. Taken together, *K. oxytoca* could be proposed as having symbiotic relationship with the mosquito as well as a likely synergist by facilitating invasion and replication of specific pathogens, an observation that has been previously reported proposed in the *Amblyomma maculatum* tick (Budachetri *et al.*, 2018).

Elizabethkingia (E.) meningoseptica and *Enterobacter hormaechei* were two of the notable bacteria species whose relative abundance increased with *D. immitis* infection. *E. meningoseptica* is a bacteria species with notable detection in several species of laboratory reared mosquitoes (Gonçalves *et al.*, 2019; Boissie` re *et al.* 2012; Rani *et al.* 2009). It has also been detected in the eggs of *Anopheles stephensi* indicating a vertical means of transmission (Ngwa *et al.*, 2013). Ngwa *et al.* (2013) also reported antiplasmodial and antimicrobial activity associated with *E. meningoseptica* in *A. stephensi*. While evaluation of antimicrobial or antiparasitic characteristics of the microbial communities detected was beyond the scope of our study, we propose that the increased abundance of *E. meningoseptica* in infected mosquitoes could 1) indirectly stem from its ability to prime the mosquito's innate immune system, hence making it possible to inhibit the filarial infection and/or 2) directly produce metabolites that inhibit *D. immitis*.

hormaechei is another bacterium that demonstrated increased abundance in *D. immitis* infection as shown in Figure 3. The genus *Enterobacter* have been proposed to aid in blood digestion in hematophagous insects due to their hemolytic activities (Gaio *et al.*, 2011). Several reports have also reported different mosquito refractoriness or susceptibility to pathogen infection in the presence of different *Enterobacter* species. Cirimotich *et al.* (2011) reported refractoriness of *Anopheles gambiae* to *Plasmodium* infection in the presence of an *Enterobacter* species. Another species of *Enterobacter* (*E. cloacae*) was also reported to express a mucin degrading Enhancin protein that breaks down the mucin component of the *A. aegypti* peritrophic matrix (Wu *et al.*, 2019) although this wasn't shown to facilitate pathogen colonization, in respect to Dengue virus infection.

Another interesting data from this study was the inverse correlation between *D. immitis* infection and microbial diversity and richness. In mosquitoes, the innate immune response is activated in the presence of invading pathogens as it was previously shown that activation of the toll pathway and production of reactive oxygen species have been reported in mosquitoes challenged with *Plasmodium* (Molina-Cruz *et al.*, 2008) and filarial nematodes (Edgerton *et al.*, 2020). These immune effectors while countering pathogen, could in extension disrupt the normal mosquito microbiome community which could explain the overall reduction in the microbial richness observed from this study as seen in Figure 4B and C. Our observation of reduced microbial richness in infected mosquitoes was in contrast to what was reported in a previous study which shows a more diverse microbiome in *Plasmodium* infected *Anopheles* mosquitoes (Bassene *et al.*, 2018).

This study fills a knowledge gap on the interaction between a mosquito vector and a filarial pathogen of veterinary significance. We were able to show that while same bacterial species were found to be present in both mosquito groups, the relative abundances of individual species changes with the infection status,

with infected mosquitoes presenting a reduced microbial richness. This indicates a likely consequence of the nematode in altering favoring or inhibiting growth of members of the bacterial community.

Declarations

Ethics Approval and Consent to participate:

Mosquitoes were blood-fed using an artificial membrane feeder. The protocol for the laboratory was approved by the Institutional Biosafety Committee.

Consent of Publication:

All authors read and approved the manuscript for publication.

Availability of data and material:

The datasets supporting the conclusion of this article are included within the article and its additional files. Raw data are available from the corresponding author upon request.

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Author contributions:

Conceived and designed the experiments: GD, SK

Performed the experiments: ASA, EN, AG, MCH, KW, SMN

Analyzed the data: ASA, GD, SK

Contributed reagents/materials/analysis tools: GD, SK

Wrote the paper: ASA, EN, AG, GD, SK

All authors have read and approved the manuscript

Competing Interests:

The authors have declared that no competing interests exist.

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Figures

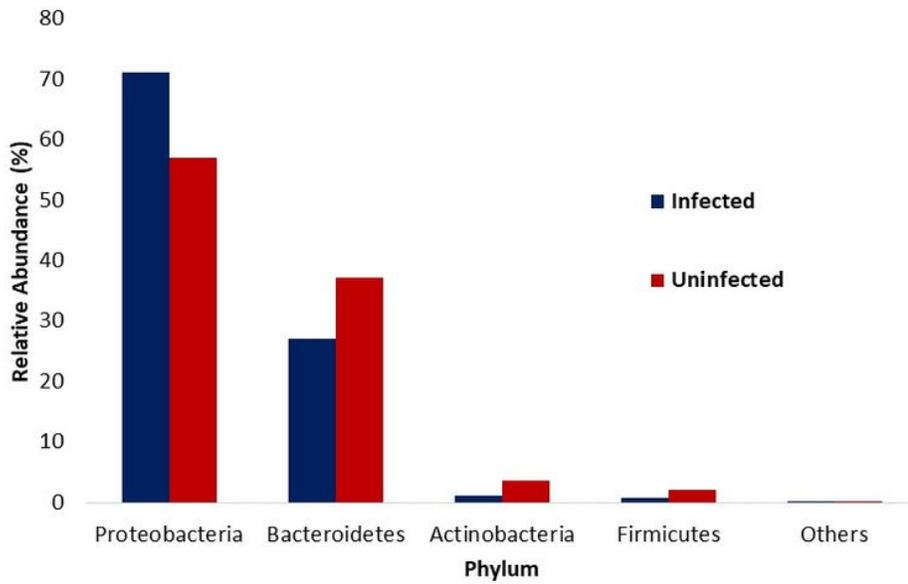


Figure 1

Relative distribution of bacteria at phylum taxonomic level in *D. immitis* infected and uninfected *Aedes aegypti* mosquitoes. Bacterial taxa with less than 1% abundances were grouped as others.

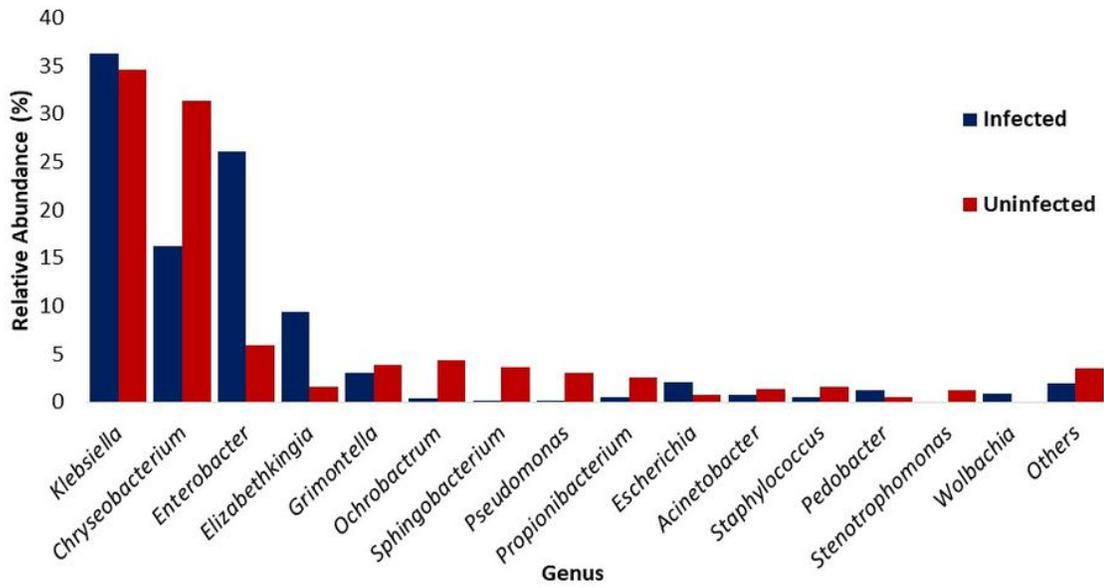


Figure 2

Relative distribution of bacteria at genus taxonomic level in *D. immitis* infected and uninfected *Aedes aegypti* mosquitoes. Bacterial taxa with less than 1% abundances were grouped as others.

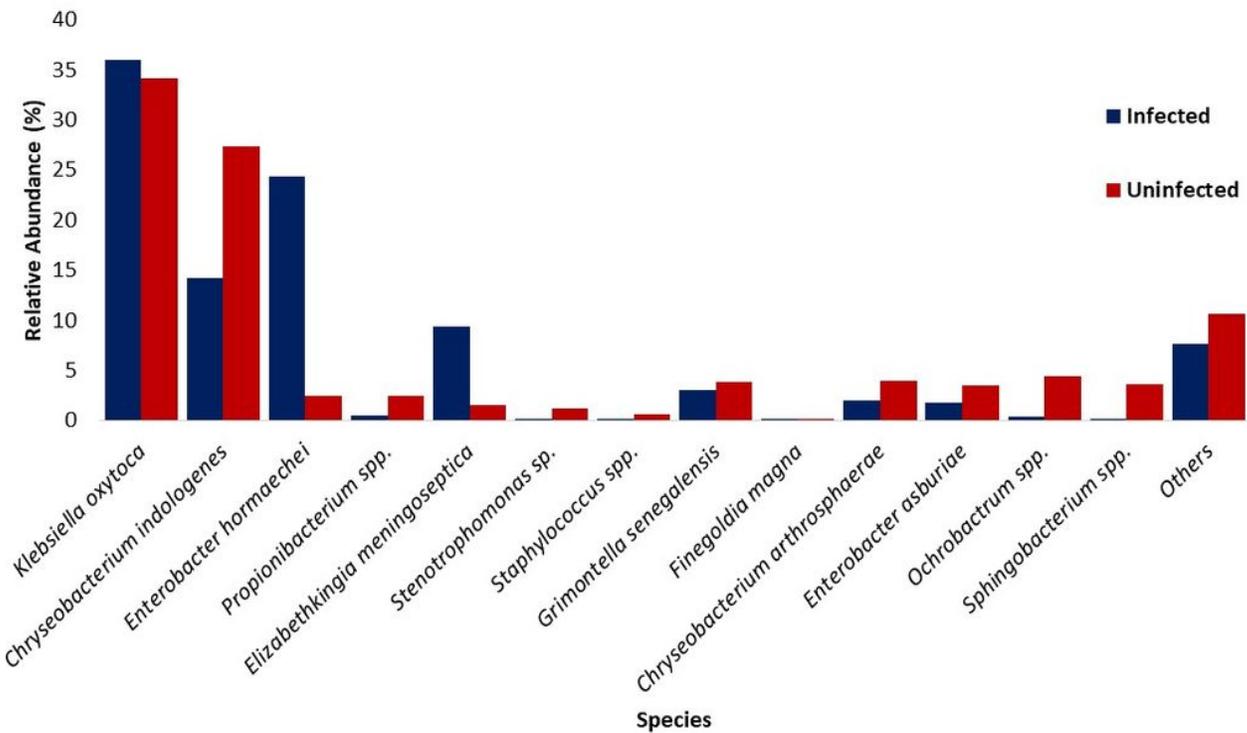


Figure 3

Relative distribution of bacteria at species taxonomic level in *D. immitis* infected and uninfected *Aedes aegypti* mosquitoes. Bacterial taxa with less than 1% abundances were grouped as others.

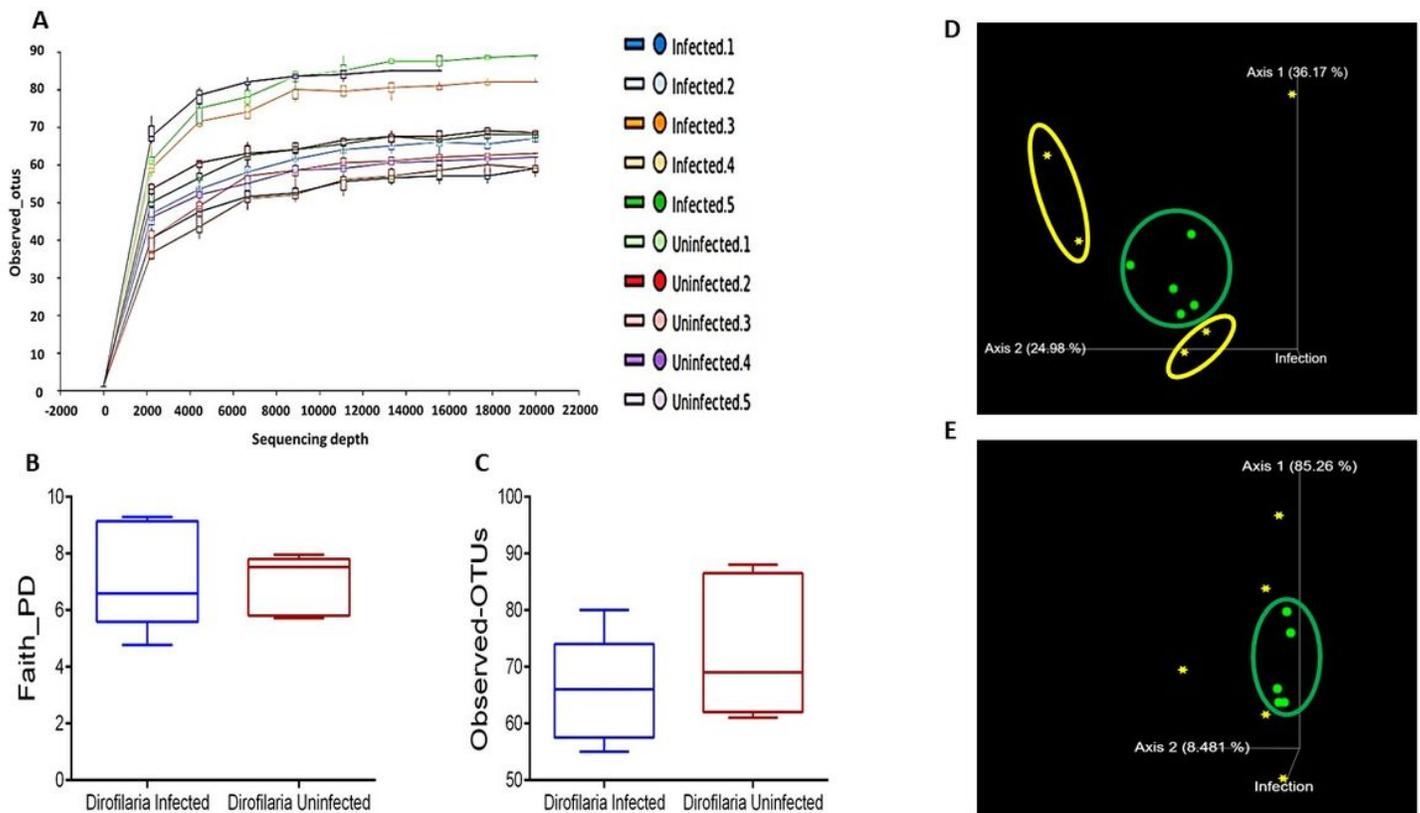


Figure 4

A), Rarefaction analysis biological replicates rarefied at a sequenced depth of 2000. Curves from each samples was allowed to reach a plateau so as to prevent trade off of rarely represented bacterial OTUs. Diversity analysis using B), Faith_phylogenetic distance and C), Observed_otus were both used as metrics to measure alpha diversity. Principal coordinate analysis (PCoA) of D), Bray_Curtis and E), Unweighted UniFrac distance matrixes as measures of beta diversity.

Supplementary Files

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- [MosquitoD.immitisMethods.png](#)