1-2017

The Microbiome of Neotropical Ticks Parasitizing On Passerine Migratory Birds

Khemraj Budachetri
University of Southern Mississippi, khem.bc@usm.edu

Jaclyn Williams
University of Southern Mississippi

Nabanita Mukherjee
University of Southern Mississippi, nabanita.mukherjee@eagles.usm.edu

Michael Sellers
University of Southern Mississippi, johnny.sellers@usm.edu

Shahid Karim
University of Southern Mississippi, Shahid.Karim@usm.edu

HHS Public Access

Author manuscript

Ticks Tick Borne Dis. Author manuscript; available in PMC 2018 January 01.

Published in final edited form as:

Ticks Tick Borne Dis. 2017 January ; 8(1): 170–173. doi:10.1016/j.ttbdis.2016.10.014.

The microbiome of neotropical ticks parasitizing on passerine migratory birds

Khemraj Budachetri, **Jaclyn Williams**, **Nabanita Mukherjee**, **Michael Sellers**, **Frank Moore**, and **Shahid Karim***

Department of Biological Sciences, University of Southern Mississippi, 118 College Drive #5018, Hattiesburg, MS 39406, USA

Abstract

Seasonal migration of passerine birds between temperate North America and tropical Central and South America is an ecological phenomenon. Migration of birds has been associated with the introduction of ectoparasites like ticks or tick-borne pathogens across the avian migration routes. In this study, the microbial diversity was determined in the ticks and bird DNA samples using 454 pyrosequencing of bacterial *16S rRNA* gene. Tick DNA samples showed the dominance of genera *Lactococcus*, *Francisella*, *Raoultella, Wolbachia* and *Rickettsia* across all the ticks, but birds DNA did not share common microbial diversity with ticks. Furthermore, *"Candidatus* Rickettsia amblyommii" infection in the 91 ticks collected off the songbirds was also quantified by qPCR assay. Interestingly, *"Candidatus* R. amblyommii" was tested positive in 24 ticks (26% infection), and infection varied from as low as three copies to thousands of copies, but bird blood samples showed no amplification. Our results provide evidence that songbirds serve as transport carrier for immature ticks, and less likely to be a reservoir for *"Candidatus* R. amblyommii".

Keywords

Rickettsia; *"Candidatus* Rickettsia amblyommii"; Microbiome; Neotropical ticks; Migratory birds

1. Introduction

Migratory birds possess the ability to travel long distances and cross ecological barriers not possible for land mammals, and are known to carry their parasites thousands of kilometers during annual migrations (Comstedt et al., 2006; Hasle, 2013; Morshed et al., 2014; Ogden et al., 2008). Ticks infesting migratory birds are capable of carrying various human pathogens, including multiple *Rickettsia* spp., *Borrelia burgdorferi*, and various *Ehrlichia* spp. (Björsdorff et al., 2001; Parola and Raoult, 2001; Rappole et al., 2000). Ground-foraging birds in particular are prone to carrying infected ticks (Elfving et al., 2010). It has been previously demonstrated that non-endemic ticks can be transported into new geographical locations, such as a *Dermacentor* species that was identified on islands off the Norwegian coast. These ticks were likely introduced by passerines, yet they were not capable of effectively settling such a cold environment (Hasle, 2013). Many songbirds travel

*Corresponding author. Shahid.Karim@usm.edu (S. Karim).

between temperate breeding areas, include the continental United States and tropical wintering areas such as Mexico and Central and South America. The migratory bird species includes the songbird species used in this study (Rappole, 1995), which were sampled at a common migratory rest site along the northern coast of the Gulf of Mexico (Moore and Kerlinger, 1987; Yong and Moore, 1997).

The microbiome study is survey of overall bacteria present in environmental samples without need of culture. The sequencing of hypervariable region of *16S rRNA* gene can identify and differentiate bacterial species (Chakravorty et al., 2007), which has been employed in next generation technology to identify thousands of bacteria simultaneously from a small quantity of samples. The structure of microbiome in body or part of body, knowing functional role in healthy individual and its association between health and disease provided important information though vast amount of data and validity of its information should be experimentally or analytically proven (Cho and Blaser, 2012; Kuczynski et al., 2011; Turnbaugh et al., 2007). The contamination at or during DNA isolation, PCR, and sequencing or even reagent used along the process could be a significant contributor and can misguide the sequencing results (Salter et al., 2014; Weiss et al., 2014). In this study, we employed the sequencing of bacterial *16S rRNA* to identify the diversities of bacteria in migratory bird blood and ticks attached with them. We focused on rickettsial agents in both bird blood and ticks to see if they have a similarity between them, at least with *Rickettsia* in overall bacterial profiling. The *"Candidatus* Rickettsia amblyommii" observed in ticks (Mukherjee et al., 2014) was quantified using specific probe based qPCR assay.

2. Materials and methods

2.1. Source of neotropical ticks and blood from birds

The bird capture, blood collection from captured birds, and tick collections were presented in early publication from the study site Johnson Bayou, Louisiana during 2009 and 2010 migration seasons (Mukherjee et al., 2014). All studies with animals were performed in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Southern Mississippi. The nylon mist net method was used for the capturing of birds. After the birds were captured, the head, neck, breast and lower surfaces of the birds were inspected visually by blowing on feathers for any tick attachments. When a tick was found, it was carefully removed using fine-tipped forceps and placed in 70% ethanol. The bird species name, band number, tick attachment site, and collection date were documented. Blood samples were taken from the 10 focal bird species representing sub-canopy/canopy, understudy and ground group (Mukherjee et al., 2014), regardless of the presence or absence of ticks, to investigate whether tickborne spotted fever group *Rickettsia* bacteria were present. The collected immature ticks were identified and described in earlier publication which were identified by using both morphological keys and molecular methods using mitochondrial *12S rDNA gene* (Beati et al., 2012; Beati and Keirans, 2001; Labruna et al., 2009; Mukherjee et al., 2014).

Quantification of "*Candidatus* Rickettsia amblyommii" in Ticks and passerine birds—The genomic DNA extracted from individual ticks and bird-blood samples by

DNeasy blood and tissue kit (Qiagen, CA) in a previous study was used. The qPCR assay was performed in 10 bird blood DNA samples, 91 tick DNA samples. But only four bird blood DNAs and only 12 larval or nymphal ticks were used for microbiome sequencing. The *"Candidatus* R. amblyommii" was quantified by using rickettsial outer membrane protein B (*rompB*) in qPCR assay (Jiang et al., 2010). Briefly, *"Candidatus* R. amblyommii" genomic DNA was used to amplify the *rompB* gene using specific primers, Ra477F (5′-GGTGCTGCGGCTTCTACATTAG-3′), Ra618R (5′-CTGAAACTTGAATAAATCCATTAGTAACAT-3′), and probe Ra532 (FAM-CGCGATCTCCTCTTACACTTGGACAGAATGCTTATCGCG-BHQ-1). In this assay, 0.5 μM of each primer, 0.4 μM probe, 3 mM magnesium chloride, and 2X TaqMan PCR master mix (Promega, Madison, WI) was used in each reaction. The *rompB* positive control (GenBank accession # FJ455415) was kindly donated by the Viral and rickettsial Diseases Department of the Naval Medical Research Center (Silver Spring, MD). The qPCR reactions were performed in a Thermal cycler (CFX96 Real time detection system, BioRad Laboratories, CA) as follows: 95°C for 2 min followed by 45 two-step cycles of 94°C for 5 s and 60°C for 30s. The number of copies/μL was calculated from standard curve prepared by using *rompB* positive control.

2.2. Bacterial diversity in neotropical ticks: 454 pyrosequencing

The pyrosequencing of tick and migratory bird DNA, and analysis of the sequencing data, was performed as previously described (Budachetri et al., 2016,2014). Briefly, tick and migratory song-bird blood DNA samples were used for bacterial tag-encoded titanium amplicon pyrosequencing (bTETAP) (Acosta-Martinez et al., 2008). The output used for analysis had an average read length of ≈450 bp with sequencing extending from the 27F 5′ GAG TTT GAT CNT GGC TCA G 3′ to 519R 5′ GTN TTA CNG CGG CKG CTG 3′ in relation to *E. coli 16S*, extending across V1 and into the V3 ribosomal region (Research and Testing Laboratory, Lubbock, TX). A single step 30 cycle PCR using HotStarTaq plus master mix kit (Qiagen, Valencia, CA) was used under the following conditions: 94°C for 3 min followed by 32 cycles of 94°C for 30 ss; 60°C for 40 s and 72°C for 1 min; and a final elongation step at 72°C for 5 min. Following PCR, all amplicon products from different samples were mixed in equal concentrations and purified using Agecourt Ampure beads (Agencourt Bioscience Corporation, MA, US).

The obtained sequences were curated to obtain Q25 sequence data, which were processed using a proprietary analysis pipeline (www.mrdnalab.com). All the sequences were trimmed to remove barcodes, primers, and short sequences less than 200 bp. Furthermore, the sequences with ambiguous base calls and homopolymer runs exceeding 6 base pairs in length were deleted (Acosta-Martinez et al., 2008; Dowd et al., 2011, 2008; Eren et al., 2011). The taxonomic level of OTU classifications were performed using BLASTn against a curated GreenGenes database (DeSantis et al., 2006). All the raw sequences obtained were submitted to GenBank as a bioproject PRJNA288373.

3. Results and discussion

The immature stages of tick species: *Amblyomma longirostre, A. nodosum*, *A. maculatum* and *Haemaphysalis juxtakochi* collected from migratory bird species: Wood thrush (*Hylocichla mustelina*), hooded warbler (*Wilsonia citrina*), indigo bunting (*Passerina cyanea*) and worm-eating warbler (*Helmitheros vermivorum*) (Fig. 1) were part of earlier publication (Mukherjee et al., 2014) and in this study we further surveyed for evidence of *"Candidatus* R. amblyommii" infections. *Amblyomma longirostre* and *A. nodosum* are neotropical ticks reported from Mexico, Brazil, Uruguay, and Argentina (Martins et al., 2014; Soares et al., 2014; Venzal et al., 2005). The survey of all possible bacteria in ticks and birds were determined by sequencing of tick and bird blood DNAs using next generation sequencing. Both sequencing and qPCR assay results revealed that the ticks and not the birds were infected with *"Candidatus* R. amblyommii" (Table 1, Fig. 1). Unexpectedly, we did not observe common bacterial genera between ticks and birds (Fig. 1).

Tick microbial studies yielded 46,499 sequences and 312 OTUs after all necessary processing was completed. Similarly, a total of 49,372 sequences, representing 213 OTUs, were obtained from the bird blood. The dominant bacterial phyla observed in the ticks were *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* (Fig. 1). The overall bird microbiota in this study was less diverse, with only two dominant bacterial phyla, *Actinobacteria* and *Proteobacteria* (Fig. 1). We did not observe a similarity in the bacterial species present between the birds' blood and the ticks infested on them. The most prevalent genera observed, with greater than 1% abundance in the ticks, were *Lactococcus, Raoultella, Wolbachia, Francisella, Propionibacterium, Ewingella, Elizabethkingia, Rickettisa. Massilia and Methylobacterium*. The bacterial genera *Francisella, Rickettsia* and *Raoultella* were observed in each of tested tick species. The migratory bird microbiome was dominated by bacteria from genera: *Microbacterium*, *Pseudomonas, Rhodococcus, Pantoea, Bacillus*, and *Sphingomonas*. However, the dominant bacterial genera among the birds did differ, as *Wilsonia citrina* (hooded warbler) had *Pseudomonas* as its dominant bacterial phyla and *Passerina cyanea* (Indio bunting) had *Pantoea* for its dominant phylum. The possible contamination of the DNA might be the reason for getting *Rhodococcus, Pseudomonas or microbacterium, Bacillus and Sphin- gomonas* in bird blood where we were expecting rickettsial reads (Weiss et al., 2014). We were not ruling out blood as sterile as there were reports of microbiota detected in bird blood (Mandal et al., 2016). Although, our results rule out possibility of migratory birds being reservoir host, birds can be rickettsiamic with *Rickettsia helvetica* (Hornok et al., 2014).

In bird blood and neotropical ticks from the passerine song birds (Table 1), we have performed quantification of *"Candidatus* Rickettsia amblyommii", though non-pathogenic but can modulate epidemiology of *R. rickettsii* at areas where both species occur (Blanton et al., 2014; Rivas et al., 2015). "*Candidatus* R. amblyommii" positive ticks represented 26% of the total (24/91) ticks tested (ranged 3-thousands copies/μL) based on *rompB* gene qPCR assay (Table 1). *A. longirostre* and *H. juxtakochi* larval and nymphal stages were observed infected with "*Candidatus* R. amblyommii". The infection of "*Candidatus* R. amblyommii" was reported in questing *H. juxtakochi* and *Amblyomma* species ticks (Castro et al., 2015). The transstadial transmission of "*Candidatus* R. amblyommii" may be the reason for

observing rickettsial agent in tick's larval and nymphal stages. We did not observe detectable level of "*Candidatus* R. amblyommii" DNA from the passerine bird blood in qPCR assay. In the United States, infection with "*Candidatus* R. amblyommii" has been associated with lone star tick, *Amblyomma americanum* (Zhang et al., 2012). In the field collected ticks, about 60% of ticks reported to carry "*Candidatus* R. amblyommii" (Moncayo et al., 2010; Zhang et al., 2012). But, the infectivity of "*Candidatus* R. amblyommii" had not been defined yet, though some report it as a possible cause of a Rocky Mountain spotted fever (RMSF)-like disease in humans (Apperson et al., 2008; Sayler et al., 2014) but its infectious ability has been denied (Blanton et al., 2014). In summary, we observed that the neotropical tick species were infected with "*Candidatus* Rickettsia amblyommii*"* carried by "*Candidatus* Rickettsia amblyommii*"* free passerine birds captured during seasonal migration from South America to the United States.

Acknowledgments

Funding was provided by the National Institute of Allergy and Infectious Diseases award # AI099919, United States Department of State award # PGA-P21049 (Pakistan-United States Science and Technology Cooperation Program) to SK. The MS-INBRE core facility is supported by the National Institute of General Medical Sciences, National Institutes of Health award (P20RR016476).

References

Acosta-Martinez V, Dowd S, Sun Y, Allen V. Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. Soil Biol Biochem. 2008; 40:2762–2770. http://dx.doi.org/10.1016/j.soilbio.2008.07.022.

Apperson CS, Engber B, Nicholson WL, Mead DG, Engel J, Yabsley MJ, Dail K, Johnson J, Watson DW. Tick-borne diseases in North Carolina: is "*Rickettsia amblyommii*"a possible cause of rickettsiosis reported as rocky mountain spotted fever? Vector Borne Zoonotic Dis. 2008; 8:597–606. http://dx.doi.org/10.1089/vbz.2007.0271. [PubMed: 18447622]

Beati L, Keirans JE. Analysis of the systematic relationships among ticks of the genera Rhipicephalus and Boophilus (Acari: Ixodidae) based on mitochondrial 12S ribosomal DNA gene sequences and morphological characters. J Parasitol. 2001; 87:32–48. http://dx.doi.org/10.1645/0022-3395(2001)087[0032:AOTSRA]2.0.CO;2. [PubMed: 11227901]

Beati L, Patel J, Lucas-Williams H, Adakal H, Kanduma EG, Tembo-Mwase E, Krecek R, Mertins JW, Alfred JT, Kelly S, Kelly P. Phylogeography and demographic history of *Amblyomma variegatum* (Fabricius) (Acari: Ixodidae), the tropical bont tick. Vector Borne Zoonotic Dis. 2012; 12:514–525. http://dx.doi.org/10.1089/vbz.2011.0859. [PubMed: 22448720]

Björsdorff A, Bergström S, Massung RF, Haemig PD, Olsen B. Ehrlichia-infected ticks on migrating birds. Emerg Infect Dis. 2001; 7:877–879. http://dx.doi.org/10.3201/eid0705.017517. [PubMed: 11747702]

Blanton LS, Mendell L, Walker DH, Bouyer DH. "*Rickettsia amblyommii*" induces cross protection against lethal rocky mountain spotted fever in a Guinea pig model. Vector Borne Zoonotic Dis. 2014; 14:557–562. http://dx.doi.org/10.1089/vbz.2014.1575. [PubMed: 25072985]

Budachetri K, Browning RE, Adamson SW, Dowd SE, Chao CC, Ching WM, Karim S. An insight into the microbiome of the *Amblyomma maculatum* (Acari: Ixodidae). J Med Entomol. 2014; 51:119–129. http://dx.doi.org/10.1603/ME12223. [PubMed: 24605461]

Budachetri K, Gaillard D, Williams J, Mukherjee N, Karim S. A snapshot of the microbiome of *Amblyomma tuberculatum* ticks infesting the gopher tortoise, an endangered species. Ticks Tick Borne Dis. 2016; 7:1225–1229. http://dx.doi.org/10.1016/j.ttbdis.2016.07.010. [PubMed: 27460902]

Castro DAM, García SGG, Dzul-Rosado K, Aguilar A, Castillo J, Gabster A, Trejos D, Zavala-Castro J, Bermúdez CSE. Questing *Amblyomma mixtum* and *Haemaphysalis juxtakochi* (Acari: ixodidae)

infected with *Candidatus* Rickettsia amblyommii from the natural environment in panama canal basin, Panama. Trop Med Health. 2015; 43:217–222. http://dx.doi.org/10.2149/tmh.2015-07. [PubMed: 26865823]

Chakravorty S, Helb D, Burday M, Connell N, Alland D. A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. J Microbiol Methods. 2007; 69:330–339. http://dx.doi.org/10.1016/j.mimet.2007.02.005. [PubMed: 17391789]

Cho I, Blaser MJ. The human microbiome: at the interface of health and disease. Nat Rev Genet. 2012; 13:260. http://dx.doi.org/10.1038/nrg3182. [PubMed: 22411464]

Comstedt P, Bergström S, Olsen B, Garpmo U, Marjavaara L, Mejlon H, Barbour AG, Bunikis J. Migratory passerine birds as reservoirs of *Lyme borreliosis* in Europe. Emerg Infect Dis. 2006; 12:1087–1095. http://dx.doi.org/10.3201/eid1207.060127. [PubMed: 16836825]

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Env Microbiol. 2006; 72:5069–5072. 72/7/5069[pii]10.1128/AEM.03006-05. [PubMed: 16820507]

Dowd SE, Callaway TR, Wolcott RD, Sun Y, McKeehan T, Hagevoort RG, Edrington TS. Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). BMC Microbiol. 2008; 8:125. http://dx.doi.org/10.1186/1471-2180-8-125 [pii]10.1186/1471-2180-8-125. [PubMed: 18652685]

Dowd SE, Delton Hanson J, Rees E, Wolcott RD, Zischau AM, Sun Y, White J, Smith DM, Kennedy J, Jones CE. Survey of fungi and yeast in polymicrobial infections in chronic wounds. J Wound Care. 2011; 20:40–47. [PubMed: 21278640]

Elfving K, Olsen B, Bergstrom S, Waldenström J, Lundkvist A, Sjöstedt A, Mejlon H, Nilsson K, Bergstrom S, Waldenstrom J, Lundkvist A, Sjostedt A, Mejlon H, Nilsson K. Dissemination of spotted fever rickettsia agents in Europe by migrating birds. PLoS One. 2010; 5:e8572. http://dx.doi.org/10.1371/journal.pone.0008572. [PubMed: 20052286]

Eren AM, Zozaya M, Taylor CM, Dowd SE, Martin DH, Ferris MJ. Exploring the diversity of *Gardnerella vaginalis* in the genitourinary tract microbiota of monogamous couples through subtle nucleotide variation. PLoS One. 2011; 6:e26732. http://dx.doi.org/10.1371/journal.pone.0026732PONE-D-11-08282 (pii). [PubMed: 22046340]

Hasle G. Transport of ixodid ticks and tick-borne pathogens by migratory birds. Front Cell Infect Microbiol. 2013; 3:48. http://dx.doi.org/10.3389/fcimb.2013.00048. [PubMed: 24058903]

Hornok S, Kováts D, Csörgő T, Meli ML, Gönczi E, Hadnagy Z, Takács N, Farkas R, Hofmann-Lehmann R. Birds as potential reservoirs of tick-borne pathogens: first evidence of bacteraemia with *Rickettsia helvetica*. Parasit. Vectors. 2014; 7:128. http://dx.doi.org/10.1186/1756-3305-7-128.

Jiang J, Yarina T, Miller MK, Stromdahl EYA, Richards AL. Molecular detection of *Rickettsia amblyommii* in *Amblyomma americanum* parasitizing humans. Vector Borne Zoonotic Dis. 2010; 10:329–340. http://dx.doi.org/10.1089/vbz.2009.0061. [PubMed: 19877809]

Kuczynski J, Lauber CL, Walters WA, Parfrey LW, Clemente JC, Gevers D, Knight R. Experimental and analytical tools for studying the human microbiome. Nat Rev Genet. 2011; 13:47–58. http://dx.doi.org/10.1038/nrg3129. [PubMed: 22179717]

Labruna MB, Onofrio VC, Beati L, Arzua M, Bertola PB, Ribeiro AF, Barros-Battesti DM. Redescription of the female, description of the male, and several new records of *Amblyomma parkeri* (Acari: Ixodidae), a South American tick species. Exp Appl Acarol. 2009; 49:243–260. http://dx.doi.org/10.1007/s10493-009-9257-z. [PubMed: 19241123]

Mandal RK, Jiang T, Al-Rubaye AA, Rhoads DD, Wideman RF, Zhao J, Pevzner I, Kwon YM. An investigation into blood microbiota and its potential association with bacterial vhondronecrosis with osteomyelitis (BCO) in broilers. Sci Rep. 2016; 6:25882. http://dx.doi.org/10.1038/srep25882. [PubMed: 27174843]

Martins TF, Labruna MB, Mangold AJ, Cafrune MM, Guglielmone AA, Nava S. Taxonomic key to nymphs of the genus Amblyomma (Acari: Ixodidae) in Argentina, with description and redescription of the nymphal stage of four Amblyomma species. Ticks Tick Borne Dis. 2014; 5:753–770. http://dx.doi.org/10.1016/j.ttbdis.2014.05.007. [PubMed: 25113984]

Moncayo AC, Cohen SB, Fritzen CM, Huang E, Yabsley MJ, Freye JD, Dunlap BG, Huang J, Mead DG, Jones TF, Dunn JR. Absence of *Rickettsia rickettsii* and occurrence of other spotted fever group rickettsiae in ticks from Tennessee. Am J Trop Med Hyg. 2010; 83:653–657. http://dx.doi.org/10.4269/ajtmh.2010.09-0197. [PubMed: 20810834]

Moore F, Kerlinger P. Stopover and fat deposition by North American wood-warblers (Parulinae) following spring migration over the Gulf of Mexico. Oecologia. 1987; 74:47–54. http://dx.doi.org/10.1007/BF00377344. [PubMed: 28310413]

Morshed, MG., Scott, JD., Fernando, K., Beati, L., Daniel, F., Geddes, G., Durden, LA., Journal, T., Geddest, G., Mazerolle, F. Migratory Songbirds Disperse Ticks Across Canada, and First Isolation of the Lyme Disease Spirochete, Borrelia burgdorferi, from the Avian Tick, *Ixodes Auritulus*. Vol. 91. The American Society of Parasitologists content in a trusted digital archi; 2014. p. 780-790.

Mukherjee N, Beati L, Sellers M, Burton L, Adamson S, Robbins RG, Moore F, Karim S. Importation of exotic ticks and tick-borne spotted fever group rickettsiae into the United States by migrating songbirds. Ticks Tick Borne Dis. 2014; 5:127–134. http://dx.doi.org/10.1016/j.ttbdis.2013.09.009S1877-959X(13)00100-3, pii. [PubMed: 24252263]

Ogden NH, Lindsay LR, Hanincová K, Barker IK, Bigras-Poulin M, Charron DF, Heagy A, Francis CM, O'Callaghan CJ, Schwartz I, Thompson RA. Role of migratory birds in introduction and range expansion of *Ixodes scapularis* ticks and of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Canada. Appl Environ Microbiol. 2008; 74:1780–1790. http://dx.doi.org/10.1128/AEM01982-07. [PubMed: 18245258]

Parola P, Raoult D. Tick-borne bacterial diseases emerging in Europe. Clin Microbiol Infect. 2001; 7:80–83. http://dx.doi.org/10.1046/j.1469-0691.2001.00200.x. [PubMed: 11298147]

Rappole JH, Derrickson SR, Hubálek Z. Migratory birds and spread of west nile virus in the western hemisphere. Emerg Infect Dis. 2000; 6:319–328. [PubMed: 10905964]

Rappole, JH. Nearctic Avian Migrants in the Neotropics: Maps, Appendices, and Bibliography. 2nd. Conservation and Research Center, National Zoological Park, Smithsonian Institution; Frant Royal, Virginia: 1995.

Rivas JJ, Moreira-Soto A, Alvarado G, Taylor L, Calderón-Arguedas O, Hun L, Corrales-Aguilar E, Morales JA, Troyo A. Pathogenic potential of a Costa Rican strain of *Candidatus Rickettsia* amblyommii in guinea pigs (Cavia porcellus) and protective immunity against *Rickettsia rickettsii*. Ticks Tick Borne Dis. 2015; 6:805–811. http://dx.doi.org/10.1016/j.ttbdis.2015.07.008. [PubMed: 26210090]

Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, Turner P, Parkhill J, Loman NJ, Walker AW. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol. 2014; 12:87. http://dx.doi.org/10.1186/s12915-014-0087-z. [PubMed: 25387460]

Sayler KA, Wamsley HL, Pate M, Barbet AF, Alleman RA. Cultivation of *Rickettsia amblyommii* in tick cells, prevalence in Florida lone star ticks (*Amblyomma americanum*). Parasit. Vectors. 2014; 7:270. http://dx.doi.org/10.1186/1756-3305-7-270.

Soares, HS., Barbieri, ARM., Martins, TF., Minervino, AHH., de Lima, JTR., Marcili, A., Gennari, SM., Labruna, MB. Ticks and rickettsial infection in the wildlife of two regions of the Brazilian Amazon. Exp Appl Acarol. 2014. http://dx.doi.org/10.1007/s10493-014-9851-6

Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature. 2007; 449:804–810. http://dx.doi.org/10.1038/nature06244. [PubMed: 17943116]

Venzal JM, Félix ML, Olmos A, Mangold AJ, Guglielmone AA. A collection of ticks (Ixodidae) from wild birds in Uruguay. Exp Appl Acarol. 2005; 36:325–331. http://dx.doi.org/10.1007/s10493-005-8433-z. [PubMed: 16132744]

Weiss S, Amir A, Hyde ER, Metcalf JL, Song SJ, Knight R. Tracking down the sources of experimental contamination in microbiome studies. Genome Biol. 2014; 15:564. http://dx.doi.org/10.1186/s13059-014-0564-2. [PubMed: 25608874]

Yong W, Moore FR. Spring stopover of intercontinental migratory thrushes along the northern coast of the gulf of Mexico. Auk. 1997; 114:263–278. http://dx.doi.org/10.2307/4089167.

Zhang X, Ren X, Norris DE, Rasgon JL. Distribution and infection frequency of *Candidatus Rickettsia* amblyommii in Maryland populations of the lone star tick (Amblyomma americanum) and culture

in an *Anopheles gambiae* mosquito cell line. Ticks Tick Borne Dis. 2012; 3:38–42. http://dx.doi.org/10.1016/j.ttbdis.2011.09.010. [PubMed: 22309857]

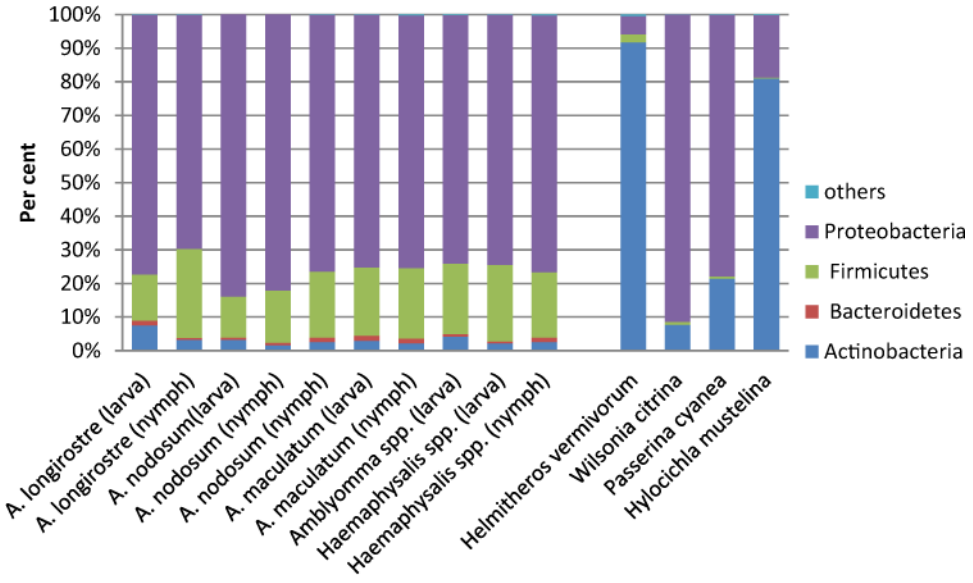

Fig. 1.

Bacterial diversity at phyla level in neotropical ticks and passerine birds. The percent a sequence reads of each bacterial phylum were presented from individual ticks and birds blood DNAs after necessary curation of sequences observed from pyrosequencing. The sequences representing particular bacterial phyla was divided by total sequences observed in each samples multiplied by 100 to get percent values. The bacterial phyla with percent values less than 1 were presented as “others”. *Amblyomma*; A.

Table 1

Quantification *of "Candidatus* Rickettsia amblyommii" in neotropical ticks from passerine migratory birds. Larva, Land Nymph, N.

Ticks	Passerine birds (Host)	*R. amblyommii* (copies/μL) (Mean ± SE) and Range of copies in Ticks
Amblyomma longirostre[L]	*Helmitheros vermivorum*	5685 ±1635(185–11095)
A. longirostre[N]	*Helmitheros vermivorum*	3
Amblyomma nodosum[N]	*Wilsonia citrina*	–
A. *nodosum*[L]	*Hylocichla mustelina*	–
Ammblyomma maculatum[L]	*Helmitheros vermivorum*	–
A. maculatum[N]	*Helmitheros vermivorum*	–
Haemaphysalis juxtakochi[L]	*Helmitheros vermivorum*	6745 ±1214(2164–21719)
H. juxtakochi[N]	*Passerina cyanea*	4