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Author(s): Emily Boothe, Matthew C. I. Medeiros, Uriel D. Kitron, Jeffrey D. Brawn, Marilyn O. Ruiz, Tony L. Goldberg, Edward D. Walker, and Gabriel L. Hamer

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Identification of Avian and Hemoparasite DNA in Blood-Engorged Abdomens of *Culex pipiens* (Diptera; Culicidae) from a West Nile Virus Epidemic region in Suburban Chicago, Illinois

EMILY BOOTHE,[1] MATTHEW C. I. MEDEIROS,[2] URIEL D. KITRON,[3] JEFFREY D. BRAWN,[4] MARILYN O. RUIZ,[5] TONY L. GOLDBERG,[6] EDWARD D. WALKER,[7] AND GABRIEL L. HAMER[1,8]

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ABSTRACT Multiple mosquito-borne parasites cocirculate in nature and potentially interact. To understand the community of parasites cocirculating with West Nile virus (WNV), we screened the bloodmeal content of *Culex pipiens* L. mosquitoes for three common types of hemoparasites. Blood-fed *Cx. pipiens* were collected from a WNV-epidemic area in suburban Chicago, IL, from May to September 2005 through 2010. DNA was extracted from dissected abdomens and subject to PCR and direct sequencing to identify the vertebrate host. RNA was extracted from the head or thorax and screened for WNV using quantitative reverse transcriptase PCR. Seventy-nine engorged females with avian host origin were screened using PCR and amplicon sequencing for filarioid nematodes, Haemosporida, and trypanosomatids. Filarioid nematodes were identified in 3.8% of the blooded abdomens, *Plasmodium* sp. in 8.9%, *Haemoproteus* in 31.6%, and *Trypanosoma* sp. in 6.3%. The sequences from these hemoparasite lineages were highly similar to sequences from birds in prior studies in suburban Chicago. Overall, 50.6% of blood-fed *Culex pipiens* contained hemoparasite DNA in their abdomen, presumably from current or prior bloodmeals. Additionally, we detected hemoparasite DNA in the blooded abdomen of three of 10 *Cx. pipiens* infected with WNV.

KEY WORDS *Culex pipiens*, American Robin, West Nile virus, avian malaria, hemoparasite

Most arthropod-borne viruses (arboviruses) in the United States that cause human disease are zoonotic, with wild and domestic animals serving as maintenance and amplification hosts (Weaver and Reisen 2010). Zoonotic mosquito-borne viruses are transmitted between mosquito vectors and wild or domestic vertebrate hosts, with occasional spillover to humans. These same wild and domestic animals are also commonly infected with other parasites, and a growing body of research suggests that parasite interactions can have important consequences on disease dynamics (Cox 2001, Pedersen and Fenton 2007, Ezenwa and Jolles 2011).

West Nile virus (WNV) is a mosquito-borne virus transmitted between *Culex* spp. mosquitoes and avian hosts and has become the most widely distributed arbovirus in the world (Kramer et al. 2008). Our previous work shows that the avian hosts, primarily American robin (*Turdus migratorius*) and house sparrow (*Passer domesticus*), responsible for the amplification of WNV in suburban Chicago are infected with other pathogens (Newman et al. 2011, Hamer et al. 2013, Hamer and Muzzall 2013, Medeiros et al. 2013) and coinfections with WNV in avian hosts result in nonrandom patterns of association (Medeiros et al. 2014). The primary avian hemoparasites observed in this study region are filarioid nematodes, haemosporida (*Plasmodium* sp. and *Haemoproteus* sp), and trypanosomatids. All these hemoparasites are vector-borne, with hematophagous dipterids as vectors and birds as definitive hosts or reservoirs (Valkiūnas 2005, Bartlett 2008).

Previous studies show that when mosquitoes coingest multiple pathogens or parasites, several mechanisms can result in enhanced or suppressed vector competence (Turell et al. 1984, Vaughan and Turell 1996a), leading to population-level impacts on arbovirus transmission (Vaughan et al. 2009, 2012). The presence of these parasites in American robins and house sparrows suggests the potential for these parasites to interact with WNV in the *Culex* spp. mosquitoes. However, few published studies have confirmed that the *Culex*

[1] Department of Entomology, Texas A&M University, 2475 TAMU, College Station, TX 77843-2475.
[2] Department of Biology, University of Missouri-St. Louis, St. Louis, One University Blvd., MO 63121.
[3] Department of Environmental Studies, Emory University, 400 Dowman Drive, Atlanta, GA 30322.
[4] Department of Natural Resources and Environmental Sciences, University of Illinois, 1102 South Goodwin Ave., Urbana, IL 61801.
[5] Department of Pathobiology, University of Illinois, 2001 S. Lincoln, Urbana, IL 61801.
[6] Department of Pathobiological Sciences, University of Wisconsin, 2015 Linden Dr., Madison, WI 53706.
[7] Department of Microbiology and Molecular Genetics, Michigan State University, 2215 Biomedical Physical Sciences East Lansing, MI 48824-4320.
[8] Corresponding author, e-mail: ghamer@tamu.edu.

mosquitoes driving WNV transmission are also exposed to these cocirculating avian hemoparasites.

The objective of the current study was to detect hemoparasite DNA in *Culex pipiens* mosquitoes that had fed on the American robin, house sparrow, and other important WNV amplification hosts. We also compare the occurrence of hemoparasites in blood-fed *Cx. pipiens* mosquitoes that are WNV positive and spatio-temporally paired WNV-negative individuals.

Materials and Methods

Sampling of Blood-fed Mosquitoes. Blood-fed *Culex* spp. mosquitoes were sampled in suburban Chicago, IL, from 2005 to 2010, from 15 seminatural and residential sites (Hamer et al. 2011). Traps consisted of CDC light traps baited with CO_2, gravid traps baited with rabbit pellet infusion, and aspirators. Blood-fed mosquitoes were identified (Andreadis et al. 2005) and stored individually at −20°C or −80°C until further processing.

Bloodmeal Analysis and WNV Detection. Blooded abdomens were removed using a sterile scalpel blade (number 11), and DNA was extracted for bloodmeal analysis using polymerase chain reaction (PCR) and sequencing (Hamer et al. 2009). Extracted DNA from the abdomens was also used for molecular identification of *Culex* spp. to confirm previous morphological identifications (Crabtree et al. 1995). RNA was extracted from the head and thorax and tested for WNV using quantitative reverse transcriptase PCR (Hamer et al. 2008b).

Parasite Screening. Blood-fed mosquitoes were selected based on their vertebrate host and WNV infection status and paired with WNV negative samples that were collected from the same trap, date, and host species. DNA extracted from selected mosquito abdomens was then screened for filarioid nematodes, Haemosporida, and trypanosomatids. To detect presence of filarioid nematodes, a 340–360 bp region of the filarial nematode mitochondrial cytochrome c oxidase subunit I (COI) gene was targeted using a nested PCR and the following primers: COIintF 5′-TGATTGGT GGTTTTGGTAA-3′, and COIintR 5′-ATAAGTAC-GAGTATCAATATC-3′ (Casiraghi et al. 2001). The PCR consisted of a 15 µl volume containing 1.5 µl of genomic DNA with 0.5 µM of each primer, 1 × Premix from the Epicentre Failsafe PCR purification kit (Epicentre Biotechnologies, Madison, WI), and 1 unit of enzyme mix. The thermal cycling profile consisted of denaturation at 94°C for 5 min, followed by 20 cycles of 94°C for 30 s, 52°C for 45 s, 72°C for 1 min, and a final extension of 72°C for 7 min. The nested PCR was performed using primers COIintF and COIintRn 5′- CATAAAAAGAAGTATTAAAATTACG -3′ (Bataille et al. 2012) and 0.5 µl of PCR product from the initial PCR. The thermal cycling conditions for the nested reaction was 94°C for 5 min, 35 cycles of 94°C for 30 s, 52°C for 45 s, 72°C for 1 min, and a final extension of 72°C for 7 min.

To detect the presence of *Plasmodium* and *Haemoproteus*, a 154 bp region of the 16 S rRNA gene was targeted using primers 343 F 5′-GCTCACG-CATCGCTTCT-3′ and 496 R 5′- GACCGGT-CATTTTCTTTG- 3′ (Fallon et al. 2003, Fecchio et al. 2013). PCRs were run at a 10 µl volume containing 1.0 µl of genomic DNA with 0.4 µM of each primer, 1 × Premix from the Epicentre Failsafe PCR purification kit, and 1 unit of enzyme mix. Cycling conditions included 2 min at 94°C, followed by 35 cycles of 94°C for 50 s, 55°C for 50 s, and 72°C for 25 s, with a final extension step at 72°C for 2 min. From samples in which positive results were detected in initial screening, a 552 bp fragment of the cytochrome b gene was amplified using a nested PCR. The outer PCR was performed using primers 3932 F 5′- GGGTTATGTAT-TACCTTGGGGTC- 3′ (Fecchio et al. 2013) and DW4R 5′ -TGTTTGCTTGGGAGCTGTAATCATA ATGTG-3′ (Perkins and Schall 2002, Fecchio et al. 2013) with 1 µl of genomic DNA. The initial cycling profile consisted of denaturation at 94°C for 4 min, followed by 35 cycles of 94°C for 20 s, 49°C for 10 s, 68°C for 45 s, and a final extension at 68°C for 3 min. One-microliter aliquot of this product was used as a template for a nested PCR with primers 413 F 5′-GTGCAACYGTTATTACTAA-3′ and 926 R 5′- CATC-CAATCCATAATAAAGCAT-3′ (Ricklefs et al. 2005) and a cycling profile consisting of 94°C for 1 min and 28 cycles of 94°C for 20 s, 52°C for 10 s, 68°C for 50 s and a final extension at 68°C for 7 min. Both PCRs consisted of a 15-µl volume containing 0.2 µM of each primer, 0.1 µg/µl BSA, 1 × Premix from the Epicentre Failsafe PCR purification kit, and 1 unit of enzyme mix.

Trypanosomes were detected by amplifying a SSU rRNA fragment targeting a 770 bp region using a nested PCR developed by Valkiūnas et al. (2011) that was modified from the Sehgal et al. (2001) protocol. Primers used for the initial PCR include Tryp763 5′-CATATGCTTGTTTCAAGGAC-3′ and Tryp1016 5′-CCCCATAATCTCCAATGGAC-3′. The PCR consisted of a 10 µl volume containing 1.0 µl of genomic DNA with 0.75 µM of each primer, 1 × Premix from the Epicentre Failsafe PCR purification kit (Epicentre Biotechnologies, Madison, WI), and 1 unit of enzyme mix. The cycling profile conditions were as follows: initial denaturation at 95°C for 5 min, followed by 5 cycles 95°C for 1 min, 45°C for 30 s, 65°C for 1 min, followed by 35 cycles of 95°C for 1 min, 50°C for 30 s, 72°C for 1 min, and then a final extension at 65°C for 10 min. The nested PCR used the primers Tryp99 5′-TCAAT-CAGACGTAATCTGCC-3′ and Tryp957 5′-CTG CTCCTTTGTTATCCCAT-3′ and consisted of a 25-µl volume containing 2.0 µl of PCR product with 0.75 µM of each primer, 1 × Premix from the Epicentre Failsafe PCR purification kit, and 1 unit of enzyme mix. The thermal cycling conditions using the second primer set were as follows: initial denaturation at 96°C for 3 min, followed by 25 cycles of 96°C for 30 s, 58°C for 1 min, 72°C for 30 s, and then a final extension at 72°C for 7 min.

Four microliters of PCR product from all reactions and one microliter of loading dye was run out on a 1% agarose gel stained with ethidium bromide and

visualized with a UV light source. Negative controls consisting of Milli-Q water were included in each batch of DNA extractions and in each PCR to monitor for contamination. Positive products were purified (ExoSAP-IT For PCR Product Clean-Up; Affymetrix). To confirm for presence of parasites, nucleotide sequences were obtained by direct sequencing of amplicon (Eton Biosciences Inc., San Diego, CA) in both the forward and reverse directions. By using a nested PCR for each hemoparasite, sequencing success was significantly higher. Sequences were removed from further analysis when lineage identity was unclear due to multiple infections or sequence quality was low. To determine if there was a difference in proportions of hemoparasite presence between WNV positive *Culex* and spatio-temporally paired WNV negative *Culex*, a Fisher exact probability test was executed using JMP Pro 11, SAS Institute Inc.

Phylogenetic Analyses. To associate parasite lineages documented in mosquito abdomens to prior lineages derived from bird blood, phylogenetic analysis of *Haemoproteus* sp., *Plasmodium* sp., and filarioid nematodes was performed. New sequences obtained from this study were compared with to homologous sequences in GenBank (http://www.ncbi.nlm.nih.gov/genbank/, accessed on 16 March 2015) using blastn for confirmation of targeted parasite DNA and aligned using ClustalW with manual correction based on chromatographs for quality control. A best-fit substitution model using Bayesian information criterion for model selection was executed in Mega 5.2.2 (Tamura et al. 2011). Phylogenetic trees were constructed using maximum likelihood method, complete deletion, General Time Reversible and discrete gamma distribution (GTR + G) and Tamura-Nei (1993) and discrete gamma distribution with invariant sites (TN93 + G + I) models for Haemosporida and filarioid nematodes, respectively, using MEGA 5.2.2 . Statistical support for phylogenetic groupings was estimated using bootstrap analysis. DNA sequences from bloodmeals with the presence of filaroid nematodes or trypanosomes were aligned with previously identified parasites from avian blood clots from studies conducted in suburban Chicago during the same field seasons (Medeiros et al. 2013). *Plasmodium* sp. and *Haemoproteus* sp. sequences were aligned with cytochrome b (cytb) sequences found in GenBank, and with sequences obtained from avian blood clot samples as described in earlier publications (Medeiros et al. 2013, 2014). Outgroups were included in the filaroid nematode analysis as described by Hamer et al. (2013).

Results

DNA in the blooded abdomens of a total of 79 *Culex pipiens* mosquitoes selected was screened for hemoparasites. Using vertebrate bloodmeal analysis, avian hosts were identified as American robins ($n = 55$), northern cardinals (*Cardinalis cardinalis*; $n = 4$), house finches (*Carpodacus mexicanus*; $n = 5$), house sparrows ($n = 13$), blue jay (*Cyanoitta cristata*; $n = 1$), and mourning dove (*Zenaida macroura*; $n = 1$). Filarioid nematode DNA was found in three blooded abdomens, *Plasmodium* sp. DNA in seven abdomens, *Haemoproteus* sp. DNA in 25 abdomens, and *Trypanosoma* sp. DNA in five abdomens (Table 1). One abdomen with the bloodmeal identified as American robin was positive for filarioid nematode and *Plasmodium* sp.

Bloodmeals from American robins were our largest sample ($n = 55$) and of these, three (5.5%) were positive for filarioid nematode, six (10.9%) for *Plasmodium* sp., 19 (34.5%) for *Haemoproteus* sp., two (3.6%) for *Trypanosoma* sp., and 30 (54.5%) for at least one of these hemoparasites. Bloodmeals from house sparrows ($n = 13$) had four (30.8%) positive for *Haemoproteus* sp. Bloodmeals from northern cardinals ($n = 4$) had one (25%) positive for *Plasmodium* sp. and two (50%) positive for *Trypanosoma* sp. Bloodmeals from house finch ($n = 5$) had two (40%) positive for *Haemoproteus* sp. and one (20%) positive for *Trypanosoma* sp. Bloodmeals from blue jay and morning dove ($n = 2$) were negative for all of the hemoparasites for which we screened.

Included in the 79 total bloodmeals were 10 individual *Culex pipiens* mosquitoes, which had previously tested WNV positive, along with spatio-temporally paired WNV negatives. Of these 10 *Culex pipiens* positive for WNV, three (30%) were positive for at least one hemoparasite. Two bloodmeals derived from American robin hosts were positive for either *Plasmodium* sp. or *Trypanosoma* sp. Another bloodmeal derived from a house finch was positive for *Haemoproteus* sp. Of the 10 paired WNV negative *Culex pipiens*, one was positive for a hemoparasite (*Trypanosoma* sp.). Statistical analysis using Fisher's exact tests showed no significant difference in the presence of hemoparasite DNA in WNV-positive *Culex pipiens versus* spatio-temporally paired negative mosquitoes (filarioid nematode, $P = 0.1$; *Plasmodium* sp., $P = 0.99$; *Haemoproteus* sp., $P = 1$; *Trypanosoma* sp., $P = 0.99$).

Parasite Identification. Two Onchocercidae sp. sequences from mosquito abdomens containing American robin blood aligned with 99.05% similarity to the Onchocercidae sp. sequence found previously in American robins from the same study region (Hamer et al. 2013, JQ867065; Fig.1 and Supp Table 1 [online only]). A third sample aligned with a 97.78% similarity and is a putative third lineage of filarioid nematodes described for suburban Chicago.

One *Plasmodium* sp. sequence derived from a mosquito abdomen consisting of American robin blood aligned with 100% similarity to the *Plasmodium* lineage CHI02PL (Medeiros et al. 2013). Two *Plasmodium* sp. sequences derived from blooded abdomens consisting of American Robin DNA aligned with 100% similarity to the *Plasmodium* lineage CHI04Pl (Medeiros et al. 2013; KC789821.1; Fig. 2). Medeiros et al. (2013) determined the *Plasmodium* lineage CHI04PL and CHI02PL are highly specialized to *T. migratorius* (Supp Table 1 [online only]).

Four *Haemoproteus* sp. sequences derived from blooded abdomens containing American robin host bloodmeals aligned with 100% similarity to the *Haemoproteus* lineages CHI23PA (Medeiros et al. 2014; KM280611; Fig. 2). Four additional sequences, three

Table 1. Number of individual mosquito abdomens with hemoparasite DNA

Host species	Onchocercidae sp. (%)	*Plasmodium* sp. (%)	*Haemoproteus* sp. (%)	*Trypanosoma* sp. (%)	Total Hemoparasite (%)
Turdus migratorius ($n = 55$)	3 (5.45)	6 (10.91)	19 (34.54)	2 (3.64)	30 (54.54)
Passer domesticus ($n = 13$)	0 (0)	0 (0)	4 (30.77)	0 (0)	4 (30.77)
Cardinalis cardinalis ($n = 4$)	0 (0)	1 (25)	0 (0)	2 (50)	3 (75)
Carpodacus mexicanus ($n = 5$)	0 (0)	0 (0)	2 (40)	1 (20)	3 (60)
Other Avian Species ($n = 2$)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total ($n = 79$)	3 (3.80)	7 (8.86)	25 (31.64)	5 (6.33)	40 (50.63)

Percentages are based upon the sample size of each species.

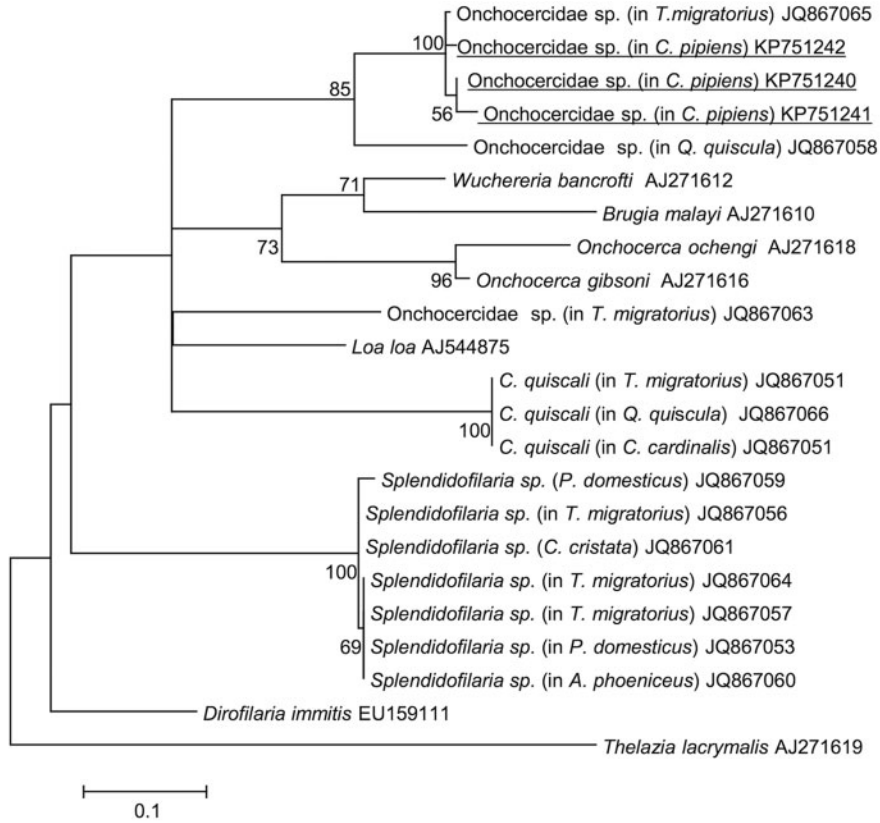

Fig. 1. Phylogenetic relationships between filarioid nematode parasites identified in blooded mosquito abdomens and other nematode parasites based on sequence variation of the mitochondrial cytochrome c oxidase subunit I gene. The tree was constructed by aligning 299 bp using Maximum likelihood method. Numbers beside branches indicate bootstrap values. Additional filarioid nematode sequences collected directly from bird blood (avian host species in parenthesis) were included in the analysis (Hamer et al. 2013). Additional sequences were obtained from NCBI GenBank for comparison and *Thelazia lacrymalis* was used as an outgroup. Numbers near branches indicate boostrap values (only values ≥50% are shown) and the scale indicates nucleotide substitutions per site.

with bloodmeals containing American robin DNA and one with house sparrow DNA, aligned with 99.74% similarity to the same *Haemoproteus* lineage CHI23PA (Medeiros et al. 2014; KM280611). Another *Haemoproteus* sp. sequence derived from a blooded abdomen containing American robin blood aligned with 99.48% similarity to CHI23PA (Medeiros et al. 2014; KM280611). Two sequences from bloodmeals consisting of American robin host DNA and two additional sequences from house sparrows host DNA aligned with 100% similarity to CHI20PA (Medeiros et al. 2014; KM280614). Three sequences derived from a mosquito abdomen containing American robin blood aligned with 99.74% similarity to CHI20PA (Medeiros et al. 2014; KM280614).

Two trypanosomatids sequences from mosquito abdomens containing DNA from a northern cardinal and a house finch aligned with a 100% similarity to the clade *Trypanosoma* sp. (Hamer et al. 2013; JQ887043.1), which was originally derived from a Northern Cardinal and most closely related to *Trypanosoma benetti* (JF7778738). Another trypanosomatid

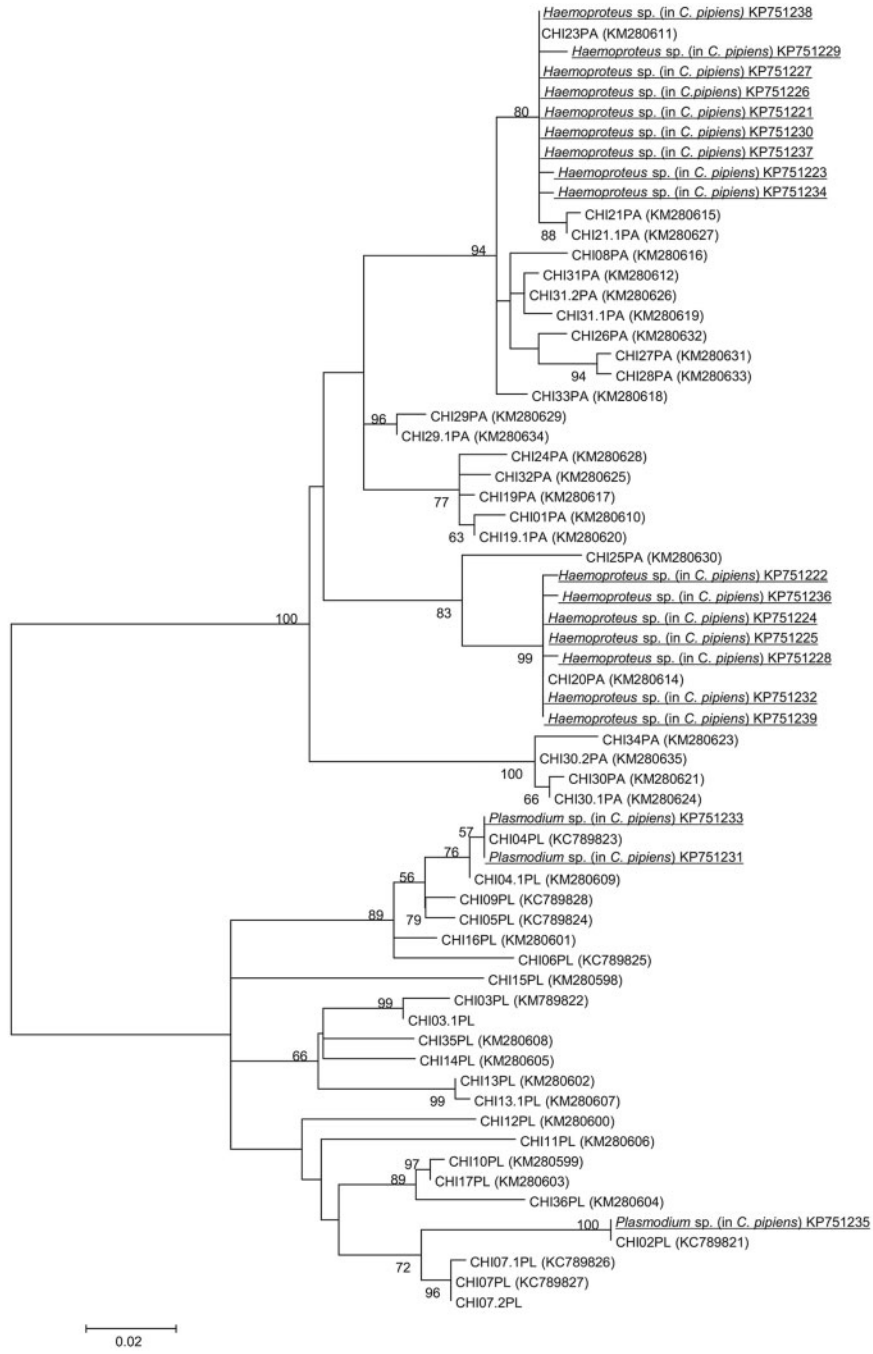

Fig. 2. Phylogenetic relationships among Haemosporidia (*Haemoproteus* sp. and *Plasmodium* sp.) parasites based on sequence variation of the cytb gene. The tree was constructed by aligning 382 bp using Maximum likelihood method. Numbers beside branches indicate bootstrap values. Additional Haemosporidia sequences collected directly from bird blood were included in the analysis (Medeiros et al. 2013).

sequence aligned per 99.07% similarity to *Trypanosoma* sp. (Hamer et al. 2013; JQ867049.1) found in a blooded abdomen with American robin DNA, and is most closely related to *Trypanosoma corvi* (AY461665).

Discussion

This study documents four avian hemoparasites that *Culex pipiens* ingests in bloodmeals while feeding on avian hosts in a "hotspot" of WNV transmission. Data shows that 54.5% of blood-fed *Culex pipiens* with

American robin blood contained hemoparasite DNA. Previous studies report prevalence of *Plasmodium* DNA in blood-fed mosquitoes that had fed on avian hosts in urban parks and zoos of Tokyo, Japan, showing the infection in *Cx. p. pallens* was 16.0% (Ejiri et al. 2011), 18.6% (Kim and Tsuda 2010), and 24.2% (Tsuda 2011). Mehus and Vaughan (2013) found that 100% of blood-fed *Cx. pipiens* and 44.4% of *Cx. tarsalis* captured were *Plasmodium* positive; however, there was no molecular evidence of filarioid nematodes or *Haemoproteus.* Our results show similar patterns.

The American robin is the most important WNV amplification host in suburban Chicago (Hamer et al. 2009, 2011) and *Cx. pipiens* is the primary enzootic and most likely bridge vector in this study region (Hamer et al. 2008a). This area of southwest suburban Chicago has been considered a "hotspot" of WNV transmission with intense epizootic and epidemic transmission occurring in 2002, 2005, 2006, 2010, and 2012 (Ruiz et al. 2004, Bertolotti et al. 2008, Hamer et al. 2008b; Illinois Department of Public Health). Results of this study show that *Cx. pipiens* are frequently exposed to several hemoparasites while taking bloodmeals from American robins and other bird species. The gonotrophic cycle of *Cx pipiens* is about 5–10 d (Hartley et al. 2012, Jones et al. 2012) and the extrinsic incubation period for WNV in *Cx. pipiens* is about 16–25 d (Anderson et al. 2008). This suggests that at least two gonotrophic cycles and two bloodmeals have occurred prior to the time that a female *Cx. pipiens* has WNV infected salivary glands, capable of delivering an infectious dose during a blood-feeding event. Moreover, we detected hemoparasite DNA in the blooded abdomen of three of 10 *Cx. pipiens* infected with WNV. Collectively, we speculate that the majority of WNV infectious female *Cx. pipiens* will have ingested at least one hemoparasite during a prior blood-feeding event.

The frequency that *Culex* mosquitoes ingest hemoparasites in bloodmeals, concurrently with WNV or prior to WNV exposure, raises the potential for parasite interactions to influence WNV transmission. Indeed, we have previously described a statistical association between WNV and *Culex flavivirus* (Newman et al. 2011). Studies in other arboviral systems have shown that various hemoparasites are capable of enhancing the dissemination and transmission of various viruses in mosquitoes. For example, *Anopheles stephensi* mosquitoes previously infected with *Plasmodium berghei* had increased transmission of Rift Valley fever virus due to the removal of the salivary gland barrier (Vaughan and Turell 1996a). Transmission of La Crosse virus by *Aedes hendersoni* increased from 8 to 72% when mosquitoes were coinfected with an avian *Plasmodium* (Paulson et al. 1992). Additionally, microfilariae, the immature stage of filarioid nematodes, are known to interact with viruses in mosquitoes through a mechanism known as microfilarial enhancement of arboviruses (Mellor and Boorman 1980, Turell et al. 1984, Vaughan and Turell 1996b, Vaughan et al. 2009). This phenomenon arises when an insect vector ingests a bloodmeal co-infected with microfilariae and an arbovirus. Microfilarial penetration of the insect midgut allows the virus to enter the hemocoel and disseminate to the salivary glands. Importantly, this process could increase vector competence as the virus bypasses the midgut barrier. Microfilarial penetration of the midgut shortens the extrinsic incubation period (Turell et al. 1987, Vaughan and Turell 1996b, Vaughan et al. 2009).

Distinguishing among avian hosts and their parasites might have been improved had we dissected DNA solely from the blood contents of the midgut (e.g., Mehus and Vaughan 2013). Prior studies have confirmed that *Haemoproteus* sp. parasite DNA is capable of being detected in the haemoceal for 17 d post ingestion (Valkiunas et al. 2013). Microfilarial DNA is detectable for at least 10 d post ingestion (Fischer et al. 2007, Erickson et al. 2009, Mehus and Vaughan 2013). The ingestion of these hemoparasites does not imply that the mosquitoes are infected, nor that they serve as the biological vector. For example, these avian microfilariae are able to penetrate the *Culex* mosquito midgut, but do not develop to the infectious L3 stage (Robinson 1971, Bartlett 2008). Valkiunas et al. (2013) show that *Haemoproteus* ookinates, but not sporozoites, remain in the mosquito haemocael for extended periods in noncompetent mosquito vectors.

In summary, we document the presence of hemoparasites in the blooded abdomens of *Cx. pipiens* mosquitoes. This study concludes that approximately half of blood-engorged *Cx. pipiens* contain hemoparasites in their blooded abdomens, and the parasite lineages are similar to those previously documented from the avian hosts in the same study region. These parasites may interact with WNV, given their presence within the vector. When calculating the vectorial capacity (VC), or daily rate at which future inoculations arise from an infective case, many parameters are included such as vector competence, extrinsic incubation period, survivorship, and host feeding rates (Dye 1992). The infection of *Culex* mosquitoes with cocirculating parasites and pathogens could influence each of these parameters and have a net effect on VC (Kenney and Brault 2013). Controlled coinfection transmission studies will be necessary to fully quantify these parameters and understand the importance of cocirculating pathogens on WNV transmission.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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