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SCIENTIFIC NOTE

HUMAN BLOODFEEDING BY THE RECENTLY INTRODUCED MOSQUITO, *AEDES JAPONICUS JAPONICUS*, AND PUBLIC HEALTH IMPLICATIONS

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ABSTRACT. Knowledge of the host-feeding behavior and extent of interactions with human hosts are important in evaluating the role and vector potential of invasive mosquitoes in transmission of native arboviruses. We collected blood-engorged females of the recently established exotic species *Aedes japonicus japonicus* from sites in New Jersey during 2000 to 2007 and identified the sources of vertebrate blood meals by sequencing portions of the cytochrome *b* gene of mitochondrial DNA. Over 1/3 (36%, $n = 36$) of the engorged mosquitoes acquired blood meals from humans. Other mammalian hosts included white-tailed deer (53%), fallow deer (5%), horse (3%), and Virginia opossum (3%). No avian, amphibian, reptilian, or mixed blood meals were identified. Our detection of a comparatively high prevalence of human bloodfeeding in *Ae. j. japonicus* in association with its local abundance, vector competence, and repeated detection of West Nile virus from field-collected specimens illustrates the potential for this invasive mosquito to serve as a ''bridge'' vector in transmission of West Nile and other mosquito-borne viruses in North America.

KEY WORDS *Aedes japonicus japonicus*, bloodfeeding behavior, West Nile virus, arboviruses

The global spread of exotic mosquito species as nuisance pests and potential vectors of diseases poses profound impacts on human health and creates serious challenges for public health and mosquito control agencies. *Aedes japonicus japonicus* (Theobald), an invasive container habitat species native to Japan, Korea, and Eastern China (Tanaka et al. 1979), was first discovered in the northeastern USA in 1998 (Peyton et al. 1999) and has rapidly spread throughout much of eastern North America and southern Canada (Andreadis et al. 2001, Fonseca et al. 2001, Sardelis and Turell 2001, Falco et al. 2002, Harrison et al. 2002, Oliver et al. 2003, Joy 2004, Reeves and Korecki 2004, Roppo et al. 2004, Young et al. 2004, Caldwell et al. 2005, Gallitano et al. 2005, Gray et al. 2005, Joy and Sullivan 2005, Larish and Savage 2005, Sames and Pehling 2005, Holman et al. 2006, Saenz et al. 2006, Thielman and Hunter 2006, Bevins 2007, Morris et al. 2007, Hughes et al. 2008).

Laboratory studies have shown that *Ae. j. japonicus* is a highly efficient vector of West Nile (WN) and St. Louis encephalitis viruses and a moderately efficient vector of eastern equine encephalitis and La Crosse viruses (Sardelis and Turell 2001; Sardelis et al. 2002a, 2002b; Sardelis et al. 2003). Furthermore, WN virus has been detected in field-collected *Ae. j. japonicus* from at least 9 different states in the northeastern and north central USA (New Hampshire, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Pennsylvania, Ohio, and Indiana) (CDC 2009), implicating this species as a ''bridge'' vector to humans.

Studies on the host-feeding behavior of *Ae. j. japonicus* are needed to define its feeding preferences in areas where it may serve as a potential vector of WN and other mosquito-borne viruses. Laboratory studies with native Asian populations indicate that this species readily feeds on chickens and mice but not on reptiles or amphibians (Miyagi 1972, Tanaka et al. 1979). However, blood meal analyses of field-collected mosquitoes in New York (Apperson et al. 2004) and Connecticut (Molaei et al. 2008) indicate that *Ae. j. japonicus* acquire blood meals exclusively from mammalian hosts, including humans. The present study examined the host-feeding habits of *Ae. j. japonicus* to extend knowledge of the behavioral ecology of this mosquito in other areas of northeastern USA and to further elucidate its role and vector potential in arbovirus transmission.

Bloodfed mosquitoes were collected from a variety of localities in rural and suburban New Jersey including farms, wood lots, waste tire and automobile disposal sites, residential backyards, and an animal park during 2000 to 2007 using New Jersey light trap collections (JW Hock Company, Gainesville, FL), grass infusion–baited

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Table 1. Number and percentage of mammalian-derived blood meals identified from *Aedes japonicus japonicus* collected in New Jersey, 2000–07.

Vertebrate host species	No.	% total	Trap type			
			GT[1]	NJ LT	CO_2 LT	ASP
White-tailed deer, *Odocoileus virginianus*	19	52.8	8	3	3	5
Human, *Homo sapiens*	13	36.1	1	—	—	12
Fallow deer, *Dama dama*	2	5.5	—	2	—	—
Horse, *Equus caballus*	1	2.8	1	—	—	—
Virginia opossum, *Didelphis virginiana*	1	2.8	—	—	1	—
Total	36	100.0	10	5	4	17

[1] GT, gravid trap; NJ LT, New Jersey light trap; CO_2 LT, CO_2-baited light trap; ASP, aspirator.

gravid traps (BioQuip Products, Inc., Rancho Dominguez, CA), carbon dioxide–baited Centers for Disease Control and Prevention (CDC) and encephalitis virus surveillance light traps (BioQuip), and aspiration from resting sites by using a backpack aspirator (BioQuip). Engorged mosquitoes were identified to species level on a chill table with the aid of a stereomicroscope using descriptive keys (Tanaka et al. 1979). Individual mosquitoes were dissected on microscope slides using flame-sterilized scalpels and forceps or disposable razor blades, and DNA was isolated from the abdominal contents of engorged mosquitoes using DNA-zol BD (Molecular Research Center, Cincinnati, OH) according to the manufacturer's recommendation with some modifications as described elsewhere (Scott 2003; Molaei et al. 2006, 2008). Isolated DNA from the mosquito blood meals served as DNA templates in subsequent polymerase chain reaction (PCR) with primers based on cytochrome *b* sequences of avian and mammalian species using previously described thermal-cycling conditions (Scott 2003; Molaei et al. 2006, 2008). The GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA) was used to perform PCR reactions and sequenced directly in cycle sequencing reactions using the sequencer, 3730xl DNA analyzer (Applied Biosystems) at the Keck Sequencing Facility, Yale University, New Haven, CT, or using ABI Prism® 3100 automated capillary genetic analyzer (Applied Biosystems) at the Biotechnology Center for Agriculture and the Environment, Rutgers University. Sequences were analyzed and annotated by using ChromasPro version 1.22 or 2.2.6 (Technelysium Pty Ltd., Tewantin, Australia) and identified by comparison to the GenBank DNA sequence database (NCBI 2008).

Analysis of the vertebrate blood meal sources for *Ae. j. japonicus* identified 5 mammalian hosts (Table 1). White-tailed deer, *Odocoileus virginianus* (Zimmermann), was the most frequently identified host (52.8% of total), followed by human, *Homo sapiens* L. (36.1%). Specimens with human-derived blood meals were collected by aspiration (92.3%, $n = 12$) and a gravid trap (7.7%), from woodlots (69.2%), waste tire disposal sites (15.4%), and residential backyards (15.4%). Other mammalian species identified included horse, *Equus caballus* (L.), and Virginia opossum, *Didelphis virginiana* (Kerr). Two mosquitoes collected within a captive wildlife facility had acquired blood meals from fallow deer, *Dama dama* L. This small facility (ca. 5 acres) houses a group of 15 fallow deer that are allowed to roam freely within the enclosure. No avian, amphibian, reptilian, or mixed blood meals were identified.

Examination of the host-feeding behavior of mosquitoes is vital to understanding their vectorial capacity in a new distribution range, particularly in instances where humans may be the preferred host and the mosquito species are capable of transmitting viruses that circulate annually and cause human disease. Our blood meal analysis of *Ae. j. japonicus* identified exclusively mammalian-derived blood meals with a high prevalence of human feeding that was notably greater than that reported for other mammalophilic mosquito species in the northeastern USA (Apperson et al. 2002, 2004; Molaei et al. 2009). This finding, in concert with the detection of WN virus from field-collected females throughout New Jersey in 8 of the last 9 years ($n = 24$ WN virus positive pools) (CDC 2008), supports a likely bridge vector role for this species in transmission of WN virus to humans in the region that has not been previously recognized. Our results also corroborate the reported attraction of *Ae. j. japonicus* to human bait stations and collection of indoor biting females in suburban and rural environs in Connecticut and New Jersey (Andreadis et al. 2001, Scott 2003).

Our results with populations in New Jersey are further consistent with the report of exclusive mammalian-derived blood meals from *Ae. j. japonicus* in New York and Connecticut, where 98% and 67% of mammalian-derived blood meals were from white-tailed deer, respectively (Apperson et al. 2004, Molaei et al. 2008). The prevalence of white-tailed deer as hosts for *Ae. j. japonicus* and other mammalophilic mosquitoes in the present and the most recent studies is likely

a function of deer abundance in the northeastern USA (Molaei et al. 2006, 2008).

The absence of avian-derived blood meals from *Ae. j. japonicus* in this and other studies from the region is enigmatic. Laboratory observations indicate that *Ae. j. japonicus* readily feeds on birds in addition to mammalian species (Miyagi 1972, Scott 2003), and vector competency studies in the USA use chickens as the blood meal source for this mosquito (Sardelis and Turell 2001; Sardelis et al. 2002a, 2002b; Sardelis et al. 2003). Furthermore, a free mating colony of *Ae. j. japonicus* established since 2000 at Rutgers University has been exclusively maintained by feeding on bobwhite quail, *Colinus virginianus* (L.) (Williges et al. 2008). The absence of avian-derived blood meals from field-collected mosquitoes could be due to the relatively small numbers and inherent experimental bias in collecting engorged mosquitoes. Samples analyzed in our study were collected at ground level, which may not harbor a large number of *Ae. j. japonicus* that feed on birds in canopies and rest near hosts (Scott 2003). Efforts in collecting this mosquito species in the canopy in New Jersey have been unsuccessful; however, Andreadis and Armstrong (2007) reported the collection of a relatively small number (16.1%, $n = 535$) of *Ae. j. japonicus* in CO_2-baited CDC light traps in tree canopies (7.6 m) when compared with light traps placed at ground level (1.5 m) in Connecticut.

Lack of avian feeding is also problematic with regard to the acquisition of WN virus by this species, since birds are viewed as the principal reservoir and amplifying hosts. Serological evidence of WN virus infection has been noted in white-tailed deer populations in New Jersey (Farajollahi et al. 2004) and Iowa (Santaella et al. 2005); however, it is not known whether they develop a sufficient viremia to infect mosquitoes and contribute to local transmission cycles. Alternatively, WN virus could be acquired from other mammals, such as eastern chipmunk, *Tamias striatus* (L.) (Platt et al. 2007), and fox squirrels, *Sciurus niger* L. (Root et al. 2006, Platt et al. 2008), for example, which have been shown to develop serum titers sufficient to infect mosquitoes in the laboratory.

Successful establishment, rapid range expansion, abundance, repeated isolation of WN virus in nature, and vector competency at rates comparable to other susceptible bridge vectors in concert with pronounced bloodfeedings on human hosts are consistent with the view that *Ae. j. japonicus* likely plays a role as a bridge vector in transmission of this virus and conceivably a number of other mosquito-borne viruses to humans and other mammals in northeastern and other regions of the USA.

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