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MOSQUITO COLLECTIONS FOLLOWING LOCAL TRANSMISSION OF *PLASMODIUM FALCIPARUM* MALARIA IN WESTMORELAND COUNTY, VIRGINIA

DANIEL STRICKMAN,[1] THOMAS GAFFIGAN,[1] ROBERT A. WIRTZ,[2] MARK Q. BENEDICT,[2] CRISTINA SALAZAR RAFFERTY,[2] RACHEL S. BARWICK[2] AND HOLLY A. WILLIAMS[2]

ABSTRACT. A 63-year-old woman from Colonial Beach, Westmoreland County, VA, was diagnosed with *Plasmodium falciparum* malaria on July 19, 1998. The woman had no history of international travel, intravenous drug use, blood transfusion, or other risk factor for contracting the disease. She seldom left the county and generally spent her evenings indoors, leading to the conclusion that she had been bitten locally by an infected mosquito. Colonial Beach is host to a population of migrant agricultural laborers from areas in which malaria occurs, but a blood survey of 89 Haitians and Mexicans failed to find *Plasmodium* parasites, specific antibodies, or clinical cases of malaria. Mosquito surveys were conducted during 2 days (July 22 and 28, 1998) with carbon-dioxide–baited light traps, larval and pupal collections, and landing collections. Thirteen species of mosquitoes were identified morphologically, including 4 potential vectors: *Anopheles crucians, An. punctipennis, An. smaragdinus* (new state record), and *An. quadrimaculatus* s.s. (new state record). Identifications of the latter 2 species were confirmed by sequencing of the ITS2 DNA region from adults reared from locally collected larvae. *Anopheles smaragdinus* was the most common biting species among the potential vectors, although *An. crucians* was the most abundant in other kinds of collections. In addition, *Ae. albopictus* was collected in Westmoreland County for the 1st time.

KEY WORDS *Plasmodium falciparum*, malaria, Virginia, *Anopheles quadrimaculatus, Anopheles smaragdinus, Aedes albopictus*

INTRODUCTION

On July 17, 1998, a 63-year-old woman living in Colonial Beach, Westmoreland County, VA, became ill with fever, myalgia, a stiff neck, and diarrhea. She was admitted to a local hospital on July 19 and promptly diagnosed and treated for malaria caused by *Plasmodium falciparum* (Welch). The diagnosis was confirmed from blood smears examined by the Biology and Diagnostics Branch, Division of Parasitic Diseases, National Center for Infectious Diseases. Because the woman had no recent history of travel outside the local area, no blood transfusion, no intravenous drug use, and no previous malaria, her infection must have been acquired locally from the bite of a mosquito. No other cases of malaria were detected in the county, despite extensive efforts to find them. The most likely source for the original introduction of *P. falciparum* was from among the large pool of migrant agricultural workers living in Colonial Beach, although interviews and blood samples of 89 people from Mexico (85) and Haiti (4) taken July 20–25, 1998, failed to find any evidence of infection.

The patient lived in a 2-story, modern, screened home, which was in excellent condition. She reported that she rarely went outside in the evening, except sometimes briefly to walk her dog. Significantly, the home was not air conditioned at night, relying on open, screened windows and ceiling fans for cooling. Close examination of the screens showed that they had adequately fine mesh (22/linear inch) and were in generally good repair. The only imperfections were small gaps between the screening frames and the sills and small tears in a few of the screens.

The neighborhood consisted of large lots (approximately 1 ha) arranged linearly along a 2-lane road. East of the lots and separating some of them were dense stands of trees, including tulip poplar (*Liriodendron tulipifera* L.), white oak (*Quercus alba* L.), sycamore (*Platanus occidentalis* L.), tupelo (*Nyssa sylvatica* Marsh), beech (*Fagus grandifolia* Ehrh.), and eastern red cedar (*Juniperus virginiana* L.). A slow-flowing stream in the woods formed an extensive area of small ponds and swamps draining into a small, tree-lined stream (Monroe Creek). Other aquatic habitats within 2 km of the house included a nearly undisturbed 2-acre pond (locally called Betty Lake) in the forest and a pond with few trees around it located adjacent to Monroe Campground. No organized mosquito abatement activity exists in the county and the state does not have central mosquito control assets to be used in case of an emergency.

MATERIALS AND METHODS

Three different kinds of mosquito collections were made between July 22 and 28, as follows: landing collections by 2 people on 2 nights from 2000 to 2200 h, collections in portable light traps (American Biophysics, Jamestown, RI) supplemented with carbon dioxide from dry ice operated on 2 nights, and collections of larvae from 12 different sites. Light trap and landing collections were

[1] Department of Entomology, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

[2] Entomology and Epidemiology Branches, Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30341-3724.

made in the backyard of the patient's home and larval collections were all made within 2 km of the home. These larvae were reared in the laboratory, preserving exuviae and adults for morphologic examination. In addition, anopheline larvae were collected on August 31, reared, and the resulting adults from the Quadrimaculatus Group retained for DNA sequence analysis of the ribosomal RNA intergenic spacer 2 (ITS2) region using a method similar to that of Cornel et al. (1996). Individual sequences and primers used for polymerase chain reaction amplification and sequencing are available from the authors (M.Q.B. or C.S.R.). The DNA sequences from the Colonial Beach collection were compared with other *Anopheles* ITS2 by alignment and clustering using the program PILEUP (Wisconsin Package Version 9.1, Genetics Computer Group, Madison, WI) with gap creation and extension penalties of 2 and 5, respectively.

Morphologic identifications of culicine species and anopheline species groups were made according to Darsie and Ward (1981). The report by Reinert et al. (1997) was used to identify members of the Quadrimaculatus Group and the work of Floore et al. (1976) was used for the Crucians Group. When associated immature forms were not available (light trap and landing collections), adults of the Quadrimaculatus Group were identified with confidence by comparison to adults from reared specimens. Because adults of species of the Crucians Group are indistinguishable, adults from traps and landing collections were assumed to be the same species as the reared specimens. All specimens were deposited at the U.S. National Museum as Walter Reed Biosystematics Unit (WRBU) Accession 1683.

Table 1. Mosquito species collected July 23–28, 1998, in the vicinity of a locally transmitted case of *Plasmodium falciparum* malaria (date of onset July 17) in Colonial Beach, Westmoreland County, VA.

Species	Number collected: Larvae/pupae[1]	Trap[2]	Landing[3]
Aedes albopictus (Skuse)	0	0	3
Ae. canadensis (Theobald)	0	0	3
Ae. triseriatus (Say)	0	1	17
Ae. vexans (Meigen)	0	1	2
Anopheles crucians Wiedemann	12	11	1
An. punctipennis (Say)	0	2	0
An. quadrimaculatus Say	7	0	1
An. smaragdinus Reinert	10	1	5
Coquillettidia perturbans (Walker)	0	0	2
Culex erraticus (Dyar and Knab)	20	11	43
Cx. salinarius Coquillett	0	6	23
Psorophora ferox (Von Humboldt)	0	0	1
Uranotaenia sapphirina (Osten Sacken)	11	1	0

[1] From 12 collections made at the margins of ponds, swamps, and streams.
[2] Portable light trap supplemented with carbon dioxide from dry ice, operated 2 nights.
[3] Two people collecting from 2000 to 2200 h for 2 nights.

RESULTS

The results of the collections (Table 1) reflected the rich variety and abundance of mosquitoes at Colonial Beach. The three most abundant biting mosquitoes were *Culex erraticus* (Dyar and Knab), *Cx. salinarius* Coq., and *Aedes triseriatus* (Say), but 8 other species were collected in just 2 evenings. Although *Anopheles smaragdinus* Reinert was the predominant biting anopheline, *An. crucians* Wied. was most abundant in larval collections and light traps. An interesting aspect of the collections was the occurrence of anophelines in every larval habitat examined. The collection of *Anopheles quadrimaculatus* Say s.s. and *An. smaragdinus* were new state records for Virginia and the collection of *Aedes albopictus* (Skuse) was a new county record (Chester G. Moore, personal communication).

The DNA sequence analysis was compared to all *Anopheles* (ITS2) sequences available to the authors, including unpublished sequences (C. Porter, personal communication). The *Anopheles* from Colonial Beach fell into 2 clusters (Fig. 1): most clustered closely with *An. quadrimaculatus* s.s. However, a more diverse group clustered with *An. smaragdinus*. These results were concordant with the morphologic identifications.

DISCUSSION

Any decision on the identity of the vector in Colonial Beach must be considered tentative, because the small number of anophelines collected precluded direct examination for sporozoites. The 2 least likely species were *Anopheles punctipennis* (Say) and *An. crucians*. Doubts about the importance of *An. punctipennis* as a malaria vector date back to the early days of malariology (Smith 1914), although the species has been proven capable of transmitting *P. falciparum* for up to 92 days (Mayne 1922). More recently, *An. punctipennis* in California was implicated as a powerful vector of *Plasmodium vivax* (Grassi and Feletti), based on its longevity and short gonotrophic cycle (Jensen et al. 1998). The scarcity of *An. punctipennis* at Colonial Beach, which is apparently typical for this species in coastal areas of the region (Vogt 1947, Gladney and Turner 1969), makes it an unlikely candidate as the local vector. Although ample records exist of naturally infected *An. crucians* (Floore et al. 1976), this species was also an unlikely vector at Colonial Beach. The scarcity of *An. crucians* in the landing collections compared to its relative abundance in

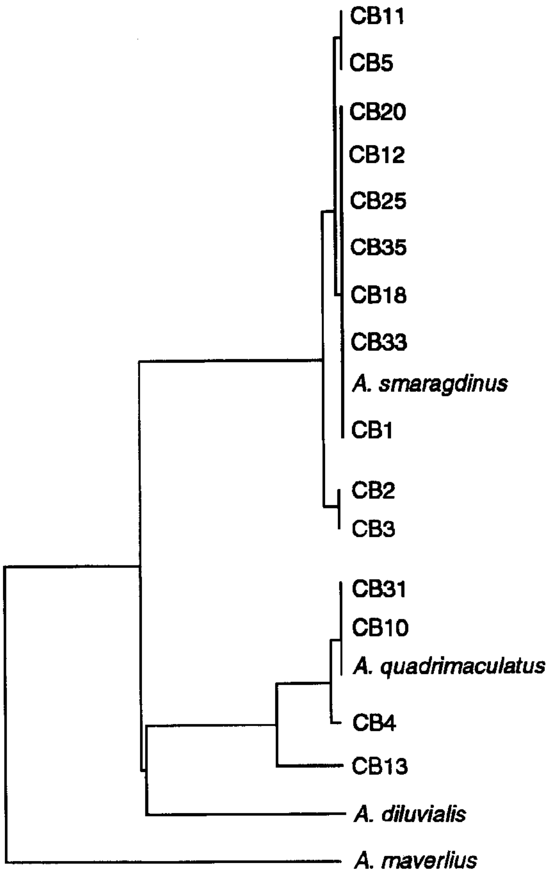

Fig. 1. Unpaired grouping with mathematical averages (UPGMA) tree of laboratory-reared *Anopheles quadrimaculatus* Group specimens from Colonial Beach. CB followed by a number is the abbreviation for Colonial Beach and the individual analyzed; species names represent known specimens sequenced for comparison.

light traps probably substantiates its lack of preference for human blood meals (Gladney and Turner 1969, Floore et al. 1976).

Anopheles quadrimaculatus s.l. has long been considered the primary vector of malaria in the eastern USA (Faust 1949, Gladney and Turner 1969). However, nothing in the current literature suggests that either *An. quadrimaculatus* s.s. or *An. smaragdinus* is the more likely malaria vector. Natural populations of these species have been observed to feed preferentially on large mammals (Apperson and Lanzaro 1991) and field-caught individuals of each species have been captured containing human blood (Jensen et al 1996). Furthermore, *An. quadrimaculatus* serves as a good experimental vector of *P. vivax* (Nayar et al. 1997). Our observation that 5 *An. smaragdinus* were captured in landing collections compared to a single *An. quadrimaculatus* might tentatively suggest that the former species was the vector. However, conditions may have been very different 3 wk before these collections, at the time the patient in Colonial Beach was probably bitten by an infected mosquito.

Malaria is not new to Virginia, having been hyperendemic in the mid 1800s. During the Civil War, Union troops suffered attack rates as high as 186.3 cases/1,000 people/year in this region. Malaria had declined dramatically by 1915, but Virginia still had 32,600 cases and a death rate of 3.0/100,000 population. Between 1929 and 1940, deaths from malaria still were recorded in 3 counties adjacent to Westmoreland County. These deaths probably represented the northernmost distribution of *P. falciparum,* but *P. vivax* was more common (Faust 1949). Local transmission of malaria has not been a widespread problem in the USA since the 1950s, with only 74 cases (59 *P. vivax,* 6 *P. malariae,* 5 *P. falciparum,* and 4 unidentified) recorded in the USA (and none in Virginia) between 1957 and 1994 (Zucker 1996).

This recent case of local transmission of *P. falciparum* in Virginia is another reminder that the vectors of malaria are still abundantly present and that modern housing does not necessarily protect the individual. Total absence of mosquito abatement resources in Westmoreland County limited the options for a public health response so that the only action possible was aggressive case detection. Fortunately, no further transmission occurred. Further research could improve the ability to prevent future transmission in Virginia by attempting to implicate the most probable vectors and by determining longevity and flight range of local anophelines.

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