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7. Plant data are from 13 censuses, from the fall of 1984 through the fall of 1989. All plants were identified, and the percent cover was measured by means of a modified point-frame method [D. W. Goodall, *Aust. J. Sci. Res. Ser. B* **5**, 1 (1952)] in permanently positioned 1-m^2 quadrats. Thirty quadrats were sampled in each large patch, four in each medium patch, and two in each small patch. Patch size differences in cumulative species counts were not significant as measured by ANOVA tests.
8. Foliar arthropods were sampled in 30 censuses, from the summer of 1985 through the winter of 1987. At each census, sweep nets were used to sample 600 m of linear transects inside patches of each size. As with the plant data, cumulative species counts did not vary significantly with patch size as measured by ANOVA tests.
9. Species evenness was calculated as

$$[(1/\Sigma p_i^2) - 1] \div [(e^{-\Sigma p_i \log p_i}) - 1]$$

where p_i is the proportional representation (relative percent cover of plants or number of individuals for arthropods) of each species. Higher values indicate more even representation of multiple species; lower values indicate dominance by fewer species [R. V. Alatalo, *Oikos* **37**, 199 (1981)].
10. Mammal species censused (total captures in parentheses) were the prairie vole, *Microtus ochrogaster* (6391); deer mouse, *Peromyscus maniculatus* (2385); cotton rat, *Sigmodon hispidus* (945); white-footed mouse, *Peromyscus leucopus* (185); and western harvest mouse, *Reithrodontomys megalotis* (46).
11. Snake species censused (total captures in parentheses) were the western yellow-bellied racer, *Coluber constrictor flaviventris* (65); osage copperhead, *Agkistrodon contortix phaeogaster* (18); red-sided garter snake, *Thamnophis sirtalis parietalis* (16); black rat snake, *Elaphe obsoleta obsoleta* (12); prairie ringneck snake, *Diadophis punctatus arnyi* (6); red milk snake, *Lampropeltis triangulum syspila* (5); timber rattlesnake, *Crotalus horridus* (1); and lined snake, *Tropidoclonium lineatum* (1).
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Isolation of Eastern Equine Encephalitis Virus from *Aedes albopictus* in Florida

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Fourteen strains of eastern equine encephalitis (EEE) virus were isolated from *Aedes albopictus* mosquitoes collected in Polk County, Florida. These are the first isolations of an arbovirus of proven public health and veterinary importance from naturally infected *Ae. albopictus* in the United States since established populations of this introduced mosquito were first discovered in 1985. The widespread distribution of *Ae. albopictus* in Florida and in other areas of the United States where EEE is endemic raises concern that this species may become an epizootic and epidemic vector of EEE virus.

Aedes albopictus, a mosquito species native to Asia, was discovered in Houston, Texas, in 1985 (*1*). It is currently established in the Western Hemisphere in 21 of the contiguous United States, Hawaii, and four states in Brazil (*2*). The probable mode of introduction into the continental United States was through the importation of used tire casings from Asia (*3*). Public health officials are concerned about the establishment and spread of this species in the United States because it is a documented vector of dengue viruses in Asia (*4*) and is an experimentally competent vector for several arboviruses (*5*).

Since the discovery of *Ae. albopictus* in the United States, the federal Centers for Disease Control (CDC) have been testing samples of *Ae. albopictus* from several states for the presence of arboviruses. This has resulted in the isolation of 16 strains of a newly recognized *Bunyavirus*, provisionally named Potosi (POT) virus, from pools of *Ae. albopictus* collected in Potosi, Missouri (*6*). In addition, the Texas Department of Health has isolated Tensaw (TEN) virus, also a *Bunyavirus*, from a pool of 38 *Ae. albopictus* mosquitoes collected on 25 July 1991 in Montgomery County, Texas (*7*). Although POT and TEN viruses are not known to cause disease in humans, these isolations suggest that *Ae. albopictus* may

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Table 1. Summary of 53,228 *Aedes albopictus* tested for virus by CDC, 1987 to 1991.

State	1987	1988	1989	1990	1991
Arkansas					1,234
Florida					9,904†
Illinois	351	4,372	361		
Indiana	429	15	72		
Louisiana	953			1,693	78
Missouri			13,720*	17,519	750
Ohio		3			
South Carolina					3
Tennessee	2	1,769			
Total	1,735	6,159	14,153	19,212	11,969

*Sixteen strains of POT virus isolated (*6*). †Fourteen strains of EEE virus and one strain of KEY virus isolated (current study).

serve as an arbovirus vector in the United States. The specific objective of the present study was to document further the potential importance of *Ae. albopictus* as an arbovirus vector in the United States by testing specimens from Florida for the presence of arboviruses and by identifying the source of blood meals from engorged specimens.

From 1986 through 1991, *Ae. albopictus* extended its range from 1 to 61 of Florida's 67 counties (*8*) and became widely distributed in Polk County, where it has been collected from 69 of 148 CDC light-trap locations. During 6 to 10 June 1991, mosquito collections were made in and around a tire dump adjacent to Route 33 approximately 14 km north of Polk City and 19 km west of Disney World. The collections were made by sweeping vegetation with a mechanical aspirator (*9*) and yielded 9393 *Ae. albopictus*. The mosquitoes were sent to the Division of Vector-Borne Infectious Diseases (DVBID), CDC, in Fort Collins, Colorado, in December 1991, where 9350 whole specimens were tested in 96 pools for virus isolation by plaque assay in Vero cell culture as described in (*10*). Forty-three blood-fed specimens were tested separately for blood meal identification as described in (*11*). The whole specimens tested for virus yielded 14 strains identified as eastern equine encephalitis (EEE) virus by the indirect fluorescent antibody test with the use of a panel of alphavirus monoclonal antibodies including EEE virus complex–specific (1B1C-4) and North American EEE virus–specific (1B5C-3) monoclonal antibodies (*12*). The virus strains were reisolated from the original mosquito pools by intracranial inoculation into 1- to 3-day-old suckling mice, and virus identification of selected strains was confirmed by a plaque-reduction neutralization test (PRNT) in Vero cell culture. These are the first reported isolations of an arbovirus of established public health importance from *Ae. albopictus* collected in the United States.

The minimum infection rate of EEE virus was 1.5 per 1000 *Ae. albopictus* tested. For comparison, the minimum EEE virus infection rate in the principal enzootic vector, *Culiseta melanura*, in west-central Florida during the month of May from 1963 to 1970 was 2.4 per 1000 tested (*13*).

An additional virus (FL91-4741) was isolated from a pool of *Ae. albopictus* from the same collection. This virus, which has been identified by PRNT as Keystone virus, a member of the California (CAL) serogroup, has not been implicated as a cause of disease in humans or domestic animals. *Aedes albopictus* was also collected in two other Florida counties during June 1991 and tested for virus as follows: Gilchrist County, 100 specimens in two pools; Marion County, 430 specimens in six pools. The results were negative. During the period 1987 to 1991, the DVBID tested 53,228 *Ae. albopictus* from the United States for virus isolation (Table 1). Three viruses have been isolated from these specimens: POT virus (6), EEE virus, and the CAL serogroup virus. These isolations, plus the isolation of TEN virus in Texas (*7*), indicate that *Ae. albopictus* is a potential vector of at least four indigenous arboviruses. The Polk County tire dump, containing approximately 3 million tires at the time the collections were made, has been closed since 1988 and currently is patrolled by security police to prevent further dumping. Because the *Ae. albopictus* population at the dump has been sampled since 1989 (*8*), the infestation is at least 3 years old. The area adjacent to Route 33 north of Polk City consists of freshwater swamps interspersed with hammocks and pastures and is inhabited by a variety of wildlife species and domestic animals. Blood meals from the 43 engorged *Ae. albopictus* collected at the tire dump were identified as follows: 31% bovine, 19% deer, 14% human, 7% raccoon, 5% rabbit, 24% unidentified mammal, and 2% passeriform bird. After removal of supernatants containing blood meal suspensions, centrifuged pellets from the 43 mosquito abdomens were each resuspended in 0.5 ml of diluent and tested for virus isolation; the results were negative.

In the United States, EEE is the rarest of the mosquito-borne arboviral encephalitides but has a high fatality rate of approximately 30% (*14*). The virus is maintained in freshwater swamp habitats in an enzootic cycle principally involving *Culiseta melanura* and a variety of avian species. Numerous mosquito species have been implicated as potential epizootic vectors (*15*). During 1991, heavy spring rains in northern Florida led to exceptionally large populations of *Cs. melanura* as well as floodwater mosquito species that serve as epizootic vectors (*16*). Consequently, Florida experienced early, widespread EEE virus activity with 70 equine cases reported by the beginning of July, the most ever reported in a season by that time (*16*). Polk County reported four EEE cases in equines with onsets in May and June. Therefore, epizootic transmission of EEE virus was occurring in Polk County during the same period that infected *Ae. albopictus* were collected at the tire dump.

Results from vector competence studies showed that an *Ae. albopictus* strain from Houston, Texas, became infected with (100%, $n = 10$) and transmitted (25 to 57%, $n = 20$) the EEE virus at 8 and 15 days, respectively, after infection (*17*). This information, coupled with the data on virus isolations from field-collected specimens associated in time and space with epizootic EEE virus activity, indicates that *Ae. albopictus* poses a threat as an epizootic and epidemic vector of EEE virus. Its opportunistic feeding habits (*11*) make it well suited for this role and may increase the risk of EEE virus transmission to humans and equines in endemic areas.

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