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Scientific Note

Culex quinquefasciatus (Diptera: Culicidae) as a potential West Nile virus vector in Tucson, Arizona: Blood meal analysis indicates feeding on both humans and birds

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Abstract

Most reports from the United States suggest *Culex quinquefasciatus* mosquitoes feed minimally on humans. Given the abundance of *C. quinquefasciatus* in residential Tucson and parts of metropolitan Phoenix, and the arrival of West Nile virus to this area, discovering the blood meal hosts of the local population is important. Using a sandwich ELISA technique, the local *C. quinquefasciatus* were found to feed on both humans and birds. This suggests they should be considered potential West Nile virus vectors.

Keywords: disease vector, mosquito, ELISA, WNV, arbovirus, *Culex quinquefasciatus*

Introduction

Laboratory tests of three different *C. quinquefasciatus* populations from California showed West Nile virus infection rates from 0 to 86% and transmission rates from 0 to 58% (Goddard *et al.* 2002). Regardless of their ability to transmit West Nile virus among birds, *C. quinquefasciatus* will only effectively vector West Nile virus to humans if the mosquitoes feed on both humans and birds. Several reports (Bohart *et al.* 1978; Irby *et al.* 1988; Reisen *et al.* 1990a; Reisen *et al.* 1990b) on feeding behavior of *C. quinquefasciatus* in the U.S. suggest that *C. quinquefasciatus* feed minimally on humans. Because the desert environment differs from most places studied, and because one U.S. report from Louisianna indicates substantial human feeding (Niebylski *et al.* 1992), determining whether *C. quinquefasciatus* feed on humans and birds in the desert southwest is important.

If a population of *C. quinquefasciatus* feed on humans and birds, then vertical transmission of West Nile virus creates an additional concern, given a rate of 3 infected/1,000 progeny for *C. quinquefasciatus* in laboratory experiments (Goddard *et al.* 2003). Because of vertical transmission, vectors include not only individual mosquitoes that first feed on an infected bird, but also some of their offspring. Hence, if an interbreeding population of mosquitoes feed on both humans and birds, even if no individual mosquito fed on both, there is still potential for transmission to humans.

Culex quinquefasciatus (Diptera: Culicidae) mosquitoes are abundant in residential Tucson and in parts of metropolitan Phoenix. Several *C. quinquefasciatus* mosquito pools in Arizona tested positive for West Nile virus in fall 2003 (Az Dept of Health 2003) and the Spring of 2004 (M. Fink, personal communication). To discover if local *C. quinquefasciatus* populations have potential to vector West Nile virus from birds to humans, blood meals of blood-fed mosquitoes caught from a residential Tucson mosquito population in summer of 2002 were analyzed.

Materials and Methods

All but three of the eighty blood fed mosquitoes reported here were captured in either Centers for Disease Control (CDC) gravid traps (John Hock model 1712) filled with straw infusion or in carbon dioxide-baited CDC light traps (operated without lights) set in residential areas of Tucson. The other three mosquitoes were obtained by aspiration of mosquitoes from a residential porch. After collection, the mosquitoes were identified to species and stored at -80° C until analysis. Mosquitoes were visually inspected for presence of a blood meal.

Blood fed mosquitoes were homogenized in phosphate buffered saline (PBS) and the pestle rinsed in PBS, yielding a final solution of 150 µl. Homogenates were centrifuged, and 5 µl of the supernatant was added to relevant wells in prepared ELISA plates.

Blood meal sources were assessed using a standard sandwich ELISA (Chow *et al.* 1993; Harlow *et al.* 1988). The four categories of blood meal distinguished were bird, dog, cat, and human. Capture antibodies were (1) affinity-purified goat anti-cat IgG, Fc Fragment specific (Jackson ImmunoResearch, 102-005-008; (2) affinity-purified rabbit anti-chicken IgY (Jackson ImmunoResearch, 303-005-008; (3) affinity-purified rabbit anti-dog IgG, Fc Fragment specific (Jackson ImmunoResearch, 304-

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005-008); (4) affinity-purified goat anti-human IgG, Fc fragment specific (Jackson ImmunoResearch 109-005-008). Conjugate antibodies were peroxidase labeled: (1) affinity-purified goat anti-cat IgG, Fc Fragment specific (Jackson ImmunoResearch, 102-035-008; (2) affinity-purified rabbit anti-chicken IgY (Jackson ImmunoResearch, 303-035-003; (3) affinity-purified rabbit anti-dog IgG, Fc Fragment specific (Jackson ImmunoResearch, 304-035-008); (4) affinity-purified goat anti-human IgG, Fc fragment specific (Jackson ImmunoResearch 109-035-008).

Samples, processed in duplicate, were considered positive if each well of the pair was above OD 0.090 at 595 nm. Samples were considered negative if below 0.045. Ambiguous samples were retested. On retesting, if the samples were not clearly positive they were presumed negative.

Results and Discussion

As shown in Figure 1, of the 84 blood meals, approximately 50% of the blood meals were human, 32% bird, 12% dog, and less than 3% cat. Included are 4 multiple blood meals: 2 human/bird; 1 dog/bird; 1 cat/bird.

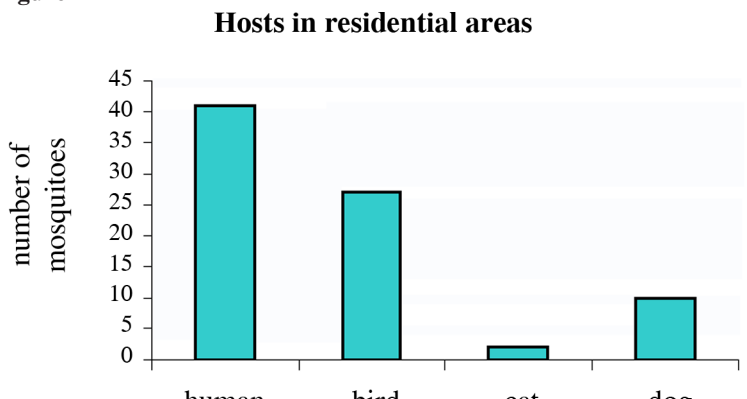
Culex quinquefasciatus is present in many areas of the world. Reports suggest considerable regional diversity exists regarding species chosen for blood meals. Early reports suggest that in North America *C. quinquefasciatus* feed predominantly on birds and less than1% of the time on human (Bohart *et al.* 1978; Reisen *et al.* 1990a). A more recent report from California found *C. quinquesfasciatus* feeds approximately equally on mammals and birds. Although testing for blood meals from humans was not included, dogs, horses, cats, and the rabbit family accounted for over 90% of the mammalian blood samples (Reisen *et al.* 1990b), suggesting that feeding on humans was less than 5% even though the sites were residential. A study of natural habitat near farms in North Carolina found no human feeding and 91% bird feeding (Irby *et al.* 1988). In contrast, analysis of blood-meal sources from *C. quinquefasciatus* in two urban sites and one wooded site in Louisianna suggest that the mosquitoes are opportunistic feeders that feed readily on humans or birds (Niebylski *et al.* 1992). Mosquitoes from a site adjacent to a dog kennel had >96% dog blood meals. More typical residential areas yielded 65-70% dog, 9-15% human, and 6-30% bird blood. A wooded area had 23-33% dog, 13-23% human, and 43-53% bird blood (Niebylski *et al.* 1992).

Studies elsewhere in the world indicate widespread variation in *C. quinquefasciatus* host choice. A residential site in Kenya had limited feeding on birds (a theoretical maximum of ~20% if all unidentified feeding was on birds; a more realistic estimate is 5-10% feeding on birds) but feeding on humans was considerable. Indoor feeding (indoor bite-rates were 700-1300 bites/man/year) was 88% human, outdoor feeding was 23% human with the majority (65%) of outdoor mammalian blood meals being unidentified mammals (Beier *et al.* 1990). In one study from southwestern Queensland, Australia, the majority of host meals were bird (~80%) regardless of habitat; 12% were human in urban areas and only 1% were human in wooded areas (Kay *et al.* 1985). A study from northern Queensland found dogs accounted for 54% of blood meals identified, humans 8.9%, and birds 29.7% (Kay *et al.* 1979). A study from southern Australia found humans to be the blood meal for 19% of the meals identified, fowl 70%, and dogs 5% (Lee *et al.* 1954). In three sites in Bangladesh, 78%, 97% and 72% of the identified blood meals were human; overall, ~93% identified meals were human (Ifteara *et al.* 1994). A maximum of <12% feeding on birds can be calculated by assuming that all non-identified meals came from birds.

These studies suggest considerable variation in host choice. Knowing the pattern of feeding of local populations may be critical for determining whether *C. quinquefasciatus* will be a significant West Nile vector. Our work, in conjunction with the literature review, suggests that, for the desert southwest, assuming that *C. quinquefasciatus* feed only minimally on humans is not justified. This is part of a larger, ongoing study to be published later in which the host species will be extended to include rabbits, and will include blood-fed mosquitoes obtained from wetland areas.

Our work indicates that, in Tucson, the species *C. quinquefasciatus* fed on both humans and birds, and that some individual mosquitoes fed on both avian and mammalian species (given that 4 of the 84 blood meals identified were positive for bird and another species). Hence, assuming the local mosquitoes are capable of being infected with West Nile virus, *C. quinquefasciatus* is potentially a significant West Nile virus vector in the urban desert southwest.

Figure 1



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