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

Margaret Zinser, Frank Ramberg, Elizabeth Willott

Entomology Department, Forbes 410, The University of Arizona, Tucson AZ 85721-0036
willott@ag.arizona.edu

Received 16 April 2004, Accepted 18 June 2004, Published 25 June 2004

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 Louisiana 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-
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 than 1% of the time on human (Bohart et al. 1978; Reisen et al. 1990a). A more recent report from California found C. quinquefasciatus 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 Louisiana 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 both 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.

Acknowledgements

We wish to thank the University of Arizona Department of Entomology for a graduate research fellowship to MZ, the Uddall Center for Studies in Public Policy for a fellowship to EW, and the University of Arizona Agricultural Experiment Station for research funds for this project. We also wish to thank Carl Pope for assistance in collecting samples.

References

Arizona Dept of Health. 2003. Fink TM, author. September 2003 ArboSurveillance Summary;Vector-Borne and Zoonotic Diseases Program, Arizona Department of Health Services, Phoenix AZ.
Beier J, Odago WO, Onyango FK, Asiago CM, Koech DK, Roberts CR. 1990. Relative abundance and blood feeding behavior of nocturnally active culicine mosquitoes in western Kenya. *Journal of the American Mosquito Control Association* 6: 207-12

Bohart RM, Washino RK. 1978. Mosquitoes of California. Berkeley: University of California, Division of Agricultural Science.

Chow E, Wirtz RA, Scott TW. 1993. Identification of blood meals in *Aedes aegypti* by antibody sandwich enzyme-linked immunosorbent assay. *Journal of the American Mosquito Control Association* 9: 196-205

Goddard LB, Roth AE, Reisen WK, Scott TW. 2002. Vector competence of California mosquitoes for West Nile virus. *Emerging Infectious Diseases* 8: http://www.cdc.gov/ncidod/EID/vol8no12/02-0536.htm

Goddard LB, Roth AE, Reisen WK, Scott TW. 2003. Vertical transmission of West Nile virus by three California *Culex* (Diptera: Culicidae) species. *Journal of Medical Entomology* 40: 743-6

Harlow E, Lane D. 1988. Antibodies: a Laboratory Manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Ifteara S, Ahmed S, Baqui MA, Ahmed TU, Ahmed RU. 1994. Host preference of culicine mosquitoes. *Bangladesh Journal of Zoology* 22: 9-16

Irby WS, Apperson CS. 1988. Hosts of mosquitoes in the coastal plain of North Carolina. *Journal of Medical Entomology* 25: 85-93

Kay BH, Boreham PFL, Williams GM. 1979. Host preferences and feeding patterns of mosquitoes (Diptera: Culicidae) at Kowanyama, Cape York Peninsula, northern Queensland. *Bulletin of Entomological Research* 69: 441-57

Kay BH, Boreham PFL, Fanning ID. 1985. Host-feeding patterns of *Culex annulirostris* and other mosquitoes (Diptera: Culicidae) at Charleville, southwestern Queensland, Australia. *Journal of Medical Entomology* 22: 529-35

Lee DJ, Clinton KJ, O’Gower AK. 1954. The blood sources of some Australian mosquitoes. *Australian Journal of Biological Science* 7: 282-301

Niebylski ML, Meek CL. 1992. Blood-feeding of *Culex* mosquitoes in an urban environment. *Journal of the American Mosquito Control Association* 8: 173-7

Reisen WK, Meyer RP, Tempelis CH, Spoehel JJ. 1990a. Mosquito abundance and bionomics in residential communities in Orange and Los Angeles counties, California. *Journal of Medical Entomology* 27: 356-7

Reisen WK, Reeves WC. 1990b. Bionomics and ecology of *Culex tarsalis* and other potential mosquito vector species. In Reeves WC, editor. *Epidemiology and control of mosquito-borne arboviruses in California, 1943-1987* 254-329. Sacramento: California Vector Control Association