Journal of the American Mosquito Control Association, 18(4):307–315, 2002

HOST FEEDING PREFERENCES OF *ANOPHELES* SPECIES COLLECTED BY MANUAL ASPIRATION, MECHANICAL ASPIRATION, AND FROM A VEHICLE-MOUNTED TRAP IN THE TOLEDO DISTRICT, BELIZE, CENTRAL AMERICA

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ABSTRACT. The host-feeding patterns of *Anopheles albimanus, Anopheles vestitipennis,* and *Anopheles punctimacula* from the Toledo District in southern Belize were studied with blood-fed females that were collected by manual aspiration, a backpack aspirator, and a vehicle-mounted trap for sampling in-flight mosquito populations. Female *An. vestitipennis* collected from both inside and outside house walls by manual aspiration tested positive for human blood meals (88 and 67%, respectively). At increasing distances from the houses, specimens of *An. vestitipennis* collected from vegetation with the backpack aspirator were equally positive for human and cattle blood (44 and 43%, respectively). In contrast, 68% of the *An. albimanus* specimens (148) collected by backpack aspiration tested positive for cattle blood. Engorged *An. vestitipennis* from vehicle-mounted trap collections tested positive for cattle (108) and human (52) blood. Almost all specimens of *An. albimanus* from these collections were positive for cow (95%). After analyzing the data from the *An. vestitipennis* samples using the feeding index, the ratio of human blood to all other bloodmeal sources showed indices greater than 1. Both *An. albimanus* and *An. punctimacula* fed mostly on cattle and rarely fed on humans. Foraging ratios for the 3 *Anopheles* species were very similar to the feeding indexes. Ratios based on data from all collection methods showed that *An. vestitipennis* feeds predominately on humans. The foraging ratios for *An. albimanus* demonstrated consistent preferences for nonhuman hosts. As with previous studies, *An. albimanus* seemed to prefer cattle and pigs to almost all other host species.

KEY WORDS Malaria, *Anopheles,* host preference, Belize, ELISA

INTRODUCTION

A key component of arthropod-borne disease epidemiology is host range of the vector species and the degree of biting contact between the suspected vector and humans. Factors that may alter man–vector contact are host availability (Washino and Tempelis 1983), seasonal changes in mosquito abundance (Charlwood et al. 1985), and sampling method (Garrett-Jones 1964).

Although malaria vectors of Central and South America have been studied, additional information is needed for several *Anopheles* species (Roberts and Andre 1994). *Anopheles albimanus* Weidemann is considered one of the most important vectors in Central America and northern South America (Faran 1980, Breeland 1980, Ramsey et al. 1994) even though this species exhibits a weak endophagic behavior (Elliott 1972, Frederikson 1993, Roberts et al. 1993, Bangs 1999, Roberts et al. 2000). Studies conducted on the feeding preference of *An. albimanus* from other regions of Central America (Garrett-Jones 1964, Breeland 1972, Garrett-Jones et al. 1980, Loyola et al. 1993) all conclude that this species is zoophagic, with a particular opportunistic feeding behavior favoring mammalian hosts. It appears to prefer feeding on large domestic animals such as horses, cattle, and pigs rather than on humans (Loyola et al. 1993).

Anopheles vestitipennis Dyar and Knab has, until recently, only been suspected of being a secondary malaria vector. It is found throughout the coastal regions of Mexico and Central America and regions of northern South America, Cuba, and Puerto Rico (Loyola et al. 1991, Mekuria et al. 1991, Marquetti et al. 1992, Padilla et al. 1992). Loyola et al. (1991) observed this species to exhibit both endophilic and endophagic behaviors. These behaviors were coupled with positive ELISA results for *Plasmodium vivax* in a native population of *An. vestitipennis* collected from Chiapas, Mexico. The positive results from studies in Mexico confirm earlier findings by Kumm and Ram (1941), who found 1 of 41 specimens of *An. vestitipennis* collected from Belize to be positive for *Plasmodium* spp. by salivary gland dissection. This species demonstrates a strong endophagic behavior in Belize (Bangs 1999, Grieco et al. 2000), which would indicate a willingness to feed on humans. Studies conducted on the feeding preference of *An. vestitipennis* are few in number. Arredondo-Jimenez et al. (1996) showed *An. vestitipennis* to more strongly prefer human hosts than *An. albimanus.* Bangs (1999) also suggested that the foraging ratios for *An. vestitipennis* indicate a preference for human hosts, while *An. albimanus* prefer cattle.

In October of 1997, a study was initiated to determine the feeding preferences of *An. albimanus* and *An. vestitipennis* in southern Belize, Central America. The objective was to assess the frequency that the 2 vectors fed on different hosts with increasing distances from human habitation. This was an attempt to better define the vector potential of

these species for malaria parasites. To accomplish this goal, a number of collection methods were employed for obtaining blood-engorged populations. These collection methods included manual aspiration from the interior and exterior of houses, backpack aspiration of vegetation, and a mobile truck trap for sampling in-flight mosquito populations. Blood-engorged specimens were then processed by the blood meal ELISA.

MATERIALS AND METHODS

In October of 1997, experimental trials were conducted to determine effective methods for collecting blood-engorged female anophelines. Three sampling methods were found to be most effective for collecting blood-engorged specimens: manual aspiration, mechanical aspiration, and a vehicle-mounted trap. All trials were conducted at Rancho Village (16°09.954'N, 88°50.529'W) in the Toledo District of southern Belize. A 1998 census estimated the population of Rancho to be 824 individuals living in approximately 166 houses with an ethnic composition of Ketchi, East Indian, and Garfuna. The village is located about 4 miles from the Gulf of Honduras and is situated on the outskirts of the town of Punta Gorda in the Toledo District, Belize. Rancho was listed among the 5 villages with the highest number of malaria cases in the Toledo District for 1997.

Manual aspiration consisted of 2 collectors searching the interior and exterior surfaces of a house for 2 h immediately after sunset. One collector searched the exterior of the house (to include the walls, thatch, stools, woodpile, bicycles, etc.), while the other collector searched all aspects of the interior of the hut (to include walls, thatch, tables, chairs, grain bags, etc.). Normal nightly activity by the occupants of the house was allowed to continue during the collection in order to sample a naturally occurring population of blood-engorged mosquitoes. Only those mosquitoes that were observed to contain a partial or full bloodmeal were collected. To eliminate collector bias, collectors switched positions (indoor/outdoor) every 10 min. Sampling of resting mosquitoes would continue in this manner for a 2-h period after sunset. This 2-h period incorporated the peak biting activity for all *Anopheles* species collected in this study. Blood-engorged mosquitoes were aspirated with a mouth aspirator and placed in a cardboard carton labeled with the location (IN or OUT) and date of collection. At the end of the collection, cotton balls soaked with chloroform were placed on top of the cartons in order to kill the mosquitoes. The mosquitoes were returned to the laboratory, where they were identified and placed in labeled Eppendorf vials. They were then placed on silica gel until they were processed using the blood meal ELISA.

Mechanical aspiration was performed with a 12-V DC backpack aspirator within a 100-m buffer zone of vegetation around the human dwellings. Specimens were collected from along tree and fence lines, walls of animal sheds, inverted canoes, fallen logs, and other thick stands of vegetation. The aspirator was operated for 5 min and the contents examined for blood-engorged mosquitoes, which were removed and placed in Eppendorf vials. The vials were labeled with information about the location, date, and area in which the mosquitoes were found. Captured specimens were returned to the laboratory, where they were identified and preserved dry on silica gel for later processing.

A vehicle-mounted trap was used to make a series of collections from June to November of 1998 on a 1-mile stretch of road near the town of Rancho. The study site was selected based on the following attributes: 1) a series of preliminary larval collections confirmed it to be adjacent to breeding sites of *Anopheles vestitipennis, An. albimanus,* and *Anopheles punctimacula,* 2) the presence of both human habitations and cattle pastures interspersed along the route, and 3) a low volume of vehicle traffic, allowing for uninterrupted collection runs.

A portable weather station (Davis Instruments, Weather Monitor II®, Haywood, CA) was affixed to a cattle gate located in the middle of the 1-mile section of road. Meteorological data, including temperature, wind speed and direction, relative humidity, and barometric pressure, were collected every 15 min to coincide with the collection intervals. The data were later downloaded to a computer database (PC-compatible Weatherlink software v4.0, Haywood, CA).

The modified vehicle-mounted trap was based on Bidlingmayer's (1974) design. The frame of the trap was constructed using sections of ½-in. PVC pipe to which a pyramid-shaped piece of green polyester netting with a mesh size of 530 mu (Bioquip Products, Gardena, CA) was attached. This pyramid-shaped netting tapered back to a plastic funnel encased in a PVC pipe coupler that had an interior diameter measurement of 4 in. The end of the funnel terminated in an opening 1 in. in diameter. The mouth of the trap measured 4 feet wide by 3 feet high. The structure was positioned so that the mouth of the trap was located 6 in. above the roof of the vehicle in line with the leading edge of the front windshield. The trap was secured to the roof by sliding the two 6-in. legs of the trap into a wooden base. The base was secured to the hood using bungee cords. The uppermost top corners were secured with nylon cord to the front of the vehicle, and the posterior end of the trap was secured in a similar fashion to the rear of the vehicle.

The collection cups consisted of pint-sized, cardboard ice cream cartons and were modified to fit the 4-in. PVC coupler. The bottom portion of the cartons were removed and replaced with two alternating pieces of dental dam with single 1-in. slits cut in their center. The center portions of the lids were removed and replaced with polyester netting

to allow for adequate airflow. The outer diameter of the cartons measured 4-in., which allowed them to be snugly fit into the PVC coupler. The constant forward motion of the vehicle created sufficient airflow to keep mosquitoes in the collection cup.

At the beginning of each sampling run, a collection cup was inserted into the rear of the trap and the vehicle moved at 10 mph down the 1-mile section of road. This took approximately 10 min. Collection runs were conducted every 15 min throughout the night beginning 1 h before sunset and continuing 1 h after sunrise. Although there does not seem to be any evidence that the headlights of the vehicle influence the collection (Bidlingmayer 1966), only the flashing hazard lights were used during the collection to minimize the possibility that headlights attract mosquitoes. At the end of each run, the collection cup was replaced with an empty cup. The contents of the cup were immediately knocked down with chloroform and emptied onto a white plate and examined. All anopheline mosquitoes were removed to labeled, 5-dram, clear styrene vials with snap tops. This examination process took about 5 min, at the end of which the next run was begun. Representative samples of nonanopheline mosquitoes also were collected. Small holes were punched in the lids and the vials were placed in a large screw-top jar containing desiccating silica gel.

At the conclusion of a nightly collection, the specimens were identified and stored for later processing. The physiological state of the specimens also was recorded (i.e., whether there was blood present in the mosquito abdomens or whether the mosquito was fully or partially gravid).

Bloodmeal analysis

All field specimens were returned to the Uniformed Services University of the Health Sciences, where they were processed in the blood meal ELISA (Chow et al. 1993). The identification of each specimen was reconfirmed by microscopic examination using a key by Wilkerson and Strickman (1990).

The sandwich ELISA used commercially available reagents (Kirkegaard and Perry Laboratories, Gaithersburg, MD) using only heavy + light (H+L) chain-specific IgG antibodies. Capture (unlabeled) and conjugate (horseradish peroxidase labeled) from 9 host species were used: human, rat, mouse, chicken, pig, cow, dog, cat, and horse. Lyophilized antisera were reconstituted in a 1:1 solution of water:glycerine to allow for storage at −20°C without freeze-thawing.

ELISA solutions

Coating solution was made using Dulbecco's (Sigma Chemical Co., St. Louis, MO) phosphate buffered saline (PBS), pH 7.0–7.4, containing 0.01% phenol red (Sigma Chemical Co.) and 0.1% thimerosal (Sigma Chemical Co.). Blocking buffer (BB) was made using 0.5% casein (Sigma Chemical Co.), boiled, and dissolved in 0.1 N NaOH. Once dissolved and cooled, coating solution was added to achieve a final desired volume and the pH was adjusted to 7.4. Wash solution contained PBS and 0.5% Tween-20 (Sigma Chemical Co.). Grinding solution was made with 0.5% Igepal (Sigma Chemical Co.) and BB. Substrate solution was a 1:1 ABTS (2,2′-azino-di[3-ethyl-benzthiazoline sulfonate (6)]) 2-component substrate (Kirkegaard and Perry Laboratories).

Abdomens with blood were removed and placed in separate 1.6-ml Eppendorf vials and labeled with an identifying number that linked it to a database by method of collection, date of collection, location of collection, and species identification. Abdomens were suspended in 50ul of grinding solution and ground using a Foredom foot-actuated grinder (Foredom Electric Co., Bethel, CT) equipped with a plastic pestle. Pestles were rinsed twice with 75ul to create a 1:200 stock dilution. Aliquots of this stock solution were then further diluted to a 1:750 working dilution. Positive controls consisted of host sera (Sigma Chemical Co.) diluted 1:300,000 in grinding solution.

The head and thorax were removed from each specimen and placed in a separate microcentrifuge tube labeled with the identification number linking it to its corresponding abdomen. Head and thorax preparations were processed separately in the sporozoite ELISA to identify possible infection with *Plasmodium vivax* (VK210), *P. vivax* (VK247), or *Plasmodium falciparum* (Wirtz et al. 1992).

All ELISA assays were performed in Costar serocluster "U" vinyl microtiter plates (Costar, Cambridge, MA). Outer wells of each plate were filled with PBS and were not used for samples in order to eliminate the potential for edge effect. Plates were read after 15 and 30 min on a Labsystems Multiskan RC plate reader (Labsystems Oy, Helsinki, Finland) at a wavelength of 414 nm. Values were considered positive if the optical density readings exceeded the mean absorbance values plus 3 times the standard deviation of the 5 negative values. Negative controls consisted of 5 unfed colony *An. albimanus* processed in the same manner as described above. All ground specimens were stored at −20°C for no more than 1 week before being tested in the ELISA.

Data analysis

A feeding index (FI) was calculated to show preference to feed on one host type over a second host type. The equation (Kay et al. 1979) for FI was

$$\mathrm{FI} = \frac{Ne/Ne'}{Ef/Ef'}$$

where

FI = the feeding index,
Ne = number of feeds on host 1,
Ne' = number of feeds on host 2,
Ef = expected proportion of feeds on host 1, and
Ef' = expected proportion of feeds on host 2.

The expected proportion is actually the number of that host present in the study area. Feeding indexes greater than 1 indicate a preference for host 1 and FI values less than 1 indicate preference for the second host. Values close to 1 indicate no host preference. This equation was calculated with an adjustment for variations in host body size, which could account for bias in determining feeding preference. This entailed multiplying the denominator of the feeding index equation by the proportional difference in body size of host 1 to host 2.

The foraging ratio (FR) was performed on the data in order to obtain the proportion of blood meals occurring for each host type in respect to all possible hosts available in the study area. The equation for FR (Hess et al. 1968) was

$$FR = \frac{N_{AE}/N_{TE}}{N_{AP}/N_{TP}}$$

where

FR = foraging ratio,
N_{AE} = number of engorged female mosquitoes containing blood from host 1,
N_{TE} = total number of engorged females,
N_{AP} = number of hosts of type 1 in the collection area, and
N_{TP} = total number of hosts of all types in the collection area.

Resulting values are again evaluated in a similar manner as those obtained in the calculation of the feeding index (i.e., values >1 = feeding preference, values <1 = feeding avoidance, and values equal to 1 = no preference).

A human blood index (HBI) was also calculated for the blood meal data. The calculation of this value is accomplished by adding the proportion of human blood meals obtained from indoors to the proportion of human blood meals obtained from outdoors and dividing by 2 (Garrett-Jones 1964). Due to the small sample size of the indoor/outdoor collections, an unweighted mean was used in the calculations.

RESULTS

A total of 1,575 blood-engorged mosquitoes were collected from all methods employed in this study. Of these, 1,298 were *Anopheles* mosquitoes. An additional 277 culicines were collected and tested.

Of the blood-engorged *Anopheles*, 852 (66.6%) were *An. vestitipennis*, 320 (25%) were *An. albimanus*, 97 (7.6%) were *An. punctimacula*, and the remaining 10 (1.0%) were *An. darlingi*. All but 9 (0.69%) tested positive for one of the animals being screened in the blood meal ELISA. Ten specimens showed signs of multiple feeding. Of these, 6 were human/cow and 1 each were human/rat, human/dog, cow/rat, and cow/dog.

A total of 30 two-hour manual aspiration collections were conducted by two collectors, resulting in a combined 120 man-hours of labor. Those collections conducted inside resulted in 2.95 blood-engorged anopheline mosquitoes per man-hour, while those collections conducted outside resulted in 1.85 blood-engorged anopheline mosquitoes per man-hour. A total of 67 two-hour backpack aspiration sessions were conducted, for a total of 134 man-hours. This equates to 4.7 blood-engorged anopheline mosquitoes being collected per man-hour. The car-top trapping was conducted on 15 different occasions for 13 h each time. This resulted in a total of 195 man-hours of collecting, with 1.85 mosquitoes collected per man-hour of effort.

The blood meal ELISA results are presented in Table 1 by method of collection and by species. Of *An. vestitipennis* collected indoors, 150 (88%) tested positive for humans, 8 (5.0%) tested positive for canine hosts, and the rest were composed of cat (5), pig (3), rat (2), and mouse (2) (Table 1). Seven specimens of *An. darlingi* were collected inside, and all tested positive for human blood.

Of the *An. vestitipennis* collected outside, 67 (62%) contained human blood, 23 contained canine blood and 9 specimens each tested positive for cow and pig. Only 3 *An. darlingi* were collected outdoors. Of these, 2 tested positive for human blood and 1 tested positive for cow blood.

The use of a backpack aspirator on vegetation within 100 m of houses resulted in the largest number of blood-fed *An. vestitipennis* (407) and *An. albimanus* (217) being collected. The *An. vestitipennis* had fed on human (180) and cow (175) in almost equal numbers. Pig blood was found in 48 of the samples and canine blood in another 4 samples. The majority of the *An. albimanus* (148) tested positive for bovine blood. Pig blood comprised the second largest category, with 40 samples testing positive, while humans and dogs made up the remaining samples, with 24 and 5 positive samples, respectively. All specimens of *An. punctimacula* tested positive for either cow (4) or pig (3) blood.

The vehicle-mounted trap resulted in the second largest number of blood-engorged *An. albimanus* and the largest number of *An. punctimacula*. The majority of *An. vestitipennis* samples tested positive for cow blood (108) and human blood (52). The remaining samples tested positive for dog and pig blood. Almost all specimens of *An. albimanus* were positive for cow (98), with only 4 and 1 testing positive for dog and pig, respectively. *Anopheles*

Table 1. Number of *Anopheles* testing positive, using the blood meal ELISA, for hosts found at the site of collection. Mosquitoes have been separated on the basis of collection method. Collections were made from October 1997–November 1998 at the Rancho site in southern Belize. Values in parentheses indicate the percentage of a species that tested positive for a particular host.

	Human	Rat	Mouse	Cow	Pig	Dog	Cat	Total
Indoor collection								
Anopheles vestitipennis	150 (88%)	2 (1%)	2 (1%)	0	3 (2%)	8 (5%)	5 (3%)	170
An. darlingi	7 (100%)	0	0	0	0	0	0	7
Outdoor collection								
Anopheles vestitipennis	67 (62%)	0	0	9 (8%)	9 (8%)	23 (22%)	0	108
An. darlingi	2 (67%)	0	0	1 (33%)	0	0	0	3
Backpack aspiration								
Anopheles vestitipennis	180 (44%)	0	0	175 (43%)	48 (12%)	4 (1%)	0	407
An. albimanus	24 (11%)	0	0	148 (68%)	40 (18%)	5 (3%)	0	217
An. punctimacula	0	0	0	4 (57%)	3 (43%)	0	0	7
Vehicle-mounted trap								
Anopheles vestitipennis	52 (31%)	0	0	108 (65%)	3 (2%)	4 (2%)	0	167
An. albimanus	0	0	0	98 (95%)	1 (1%)	4 (4%)	0	103
An. punctimacula	1 (1%)	0	0	87 (97%)	0	2 (2%)	0	90

punctimacula also tested high for bovine blood (87), with the rest testing positive for canine (2) and human (1) blood.

The feeding indices for *An. vestitipennis, An. albimanus,* and *An. punctimacula* are shown in Table 2, 3, and 4. The first value displayed in each feeding-index column is the standard feeding index that has not been adjusted for any factors associated with host or vector populations. The second value, displayed in parentheses, is the feeding index that has been adjusted for the size difference ratio between humans and the host in question. For this analysis, an estimate was made of individual host size based on average weight across the population. Using this estimate, cattle were taken to be 5 times the size of a human, a pig was taken to be the same size as a human, and a dog was taken to be 3 times smaller than a human.

The foraging ratios for all anophelines are shown in Table 5. No *An. albimanus* or *An. punctimacula* were collected from the interior or exterior aspects of the house and therefore no foraging ratios could be calculated for these species from human habitations. In collections where these 2 species were obtained, the preferential foraging ratios for *An. albimanus* were 2.3 for cattle and 2.0 for pigs from the backpack aspiration and 3.2 for cattle in the car-trap collection. The foraging ratios calculated for

Table 2. Feeding indices for *Anopheles vestitipennis* based on method of collection of blood-engorged specimens. Collections were made from October 1997 to November of 1998 in southern Belize.

Collection method	Number of blood-engorged mosquitoes					Feeding index			
	Human	Cow	Pig	Dog	Cat	Human: cow	Human: pig	Human: dog	Human: cat
Manual aspiration, indoor	150	0	3	8	5	—	25.4 (25.4)[1]	9.4 (3.125)[2]	5.0
Manual aspiration, outdoor	67	9	9	23	0	24.8 (124)[3]	3.72 (3.72)[1]	1.45 (0.485)[2]	—
Backpack aspiration	180	175	48	4	0	3.43 (17.17)[3]	1.88 (1.88)[1]	22.5 (7.5)[2]	—
Vehicle-mounted trap	52	108	3	4	0	.91 (4.57)[3]	9.9 (9.9)[1]	4.95 (1.65)[2]	

[1] Adjustment for the relative difference in size between human and pig hosts was taken to be in a 1:1 ratio (1.0) for the feeding index calculation.

[2] Adjustment for the relative difference in size between human and dog hosts was taken to be in a 1:0.3 ratio (3.0) for the feeding index calculation.

[3] Adjustment for the relative difference in size between human and cow hosts was taken to be in a 1:5.0 ratio (0.2) for the feeding index calculation.

Table 3. Feeding indices for *Anopheles albimanus* based on method of collection of blood-engorged specimens. Collections were made from October 1997 to November of 1998 in southern Belize.

Collection method	Number of blood-engorged mosquitoes				Feeding index		
	Human	Cow	Pig	Dog	Cow: human	Cow: pig	Cow: dog
Backpack aspiration	24	148	40	5	1.85	0.55	4.44
Vehicle-mounted trap	0	98	1	4	—	29	4.9

Table 4. Feeding indices for *Anopheles punctimacula* based on method of collection of blood-engorged specimens. Collections were made from October 1997 to November of 1998 in southern Belize.

Collection method	Number of blood-engorged mosquitoes				Feeding index		
	Human	Cow	Pig	Dog	Cow: human	Cow: pig	Cow: dog
Backpack aspiration	0	4	3	0	—	0.40	—
Vehicle-mounted trap	1	87	0	2	45.8	—	5.44

this species resulted in a feeding avoidance with respect to humans and dogs (0.73 and 0.33, respectively) for samples from backpack aspiration and resulted in a feeding avoidance with respect to pigs and dogs (0.1 and 0.65, respectively) for samples from the car-trap collection. *Anopheles punctimacula* showed a preference for both cattle and pigs in the samples obtained by backpack aspiration and a preference for cattle in samples from the car-trap collection. A feeding avoidance was indicated for human and dog hosts for this mosquito species.

The foraging ratios conducted on the blood meal data for *An. vestitipennis* showed a strong feeding preference for human blood from all samples collected (indoor, 7.5; outdoor, 5.3; backpack, 3.76; car-top trap, 2.0). This species also showed a preference for cattle in the car-top collection (2.2) and dogs in the outdoor collection (3.3). Virtually no preference was shown for pigs in either the outdoor collection or the backpack collection. There also appeared to be no preference for dogs in the indoor collection and cattle in the backpack collection. A feeding avoidance was shown for pigs (0.6) and cats (0.6) in the indoor collection, cattle (0.2) in the outdoor collection, dogs (0.16) in the backpack aspiration, and pigs (0.2) and dogs (0.4) in the car-trap collection.

The human blood indices (HBI) for *An. vestitipennis* were calculated separately from the indoor/outdoor collections and for the backpack and car-trap collections. The HBI for the indoor/outdoor collections was calculated as 75%. The HBI for the backpack and car-trap collection was calculated at 37.5%.

The head and thorax from all blood-engorged specimens were tested singularly in the sporozoite ELISA, and none of the samples tested positive for *P. vivax* (VK210), *P. vivax* (VK247), or *P. falciparum.*

DISCUSSION

The use of the various collection techniques described in the Methods section resulted in blood-engorged samples being collected by only 3 methods: the indoor/outdoor resting collection, the backpack aspiration of resting mosquitoes, and the vehicle-mounted trap collections of in-flight mosquitoes. The backpack aspiration of vegetation around the houses was by far the most productive method of collecting blood-engorged *Anopheles.* In numbers collected per hour of effort, the car-top trap was the least effective method. The raw numbers indicate that this method produced the second largest number of blood-engorged *Anopheles* specimens but also required the most man-hours. Approximately 1.85 mosquitoes were collected in the car trap per man-hour. Manual aspiration outdoors was less productive than the indoor collection due primarily to the larger number of outdoor resting sites (i.e., nearby vegetation) allowing greater dispersion of blood-engorged specimens. In contrast, blood-engorged females rested on some aspect of the interior of the hut and were more easily located and collected by hand aspiration.

One would expect that the proportion of mosquitoes containing human blood would be greater inside and near houses. This appears to be the case for *An. vestitipennis.* Of females collected on inner walls, 88% contained human blood, compared with 62% that were positive for human blood on the outside walls. Moving slightly farther away from the houses, 44% of females collected with the backpack aspirator from vegetation contained human blood. As the radius of collection is extended out from the houses, the human positivity rate declined, but for *An. vestitipennis,* still remained fairly high. One thing that is very clear is that *An. albimanus* is not found in the immediate vicinity of the house. This is indicative of *An. albimanus'* weak endophagic behavior.

The vehicle-mounted trap was the most unbiased collection method in terms of distance from human habitation as well as sampling in-flight mosquito populations. Again, the percentage of *An. vestitipennis* feeding on humans dropped to 31% of those samples tested. Although the percentage feeding on cattle is much higher (65%), there still remain a large number of *An. vestitipennis* feeding on humans. The evidence of feeding on cattle is more extreme with *An. albimanus.* A comparison cannot be made for the indoor/outdoor manual aspiration

Table 5. Foraging ratios for anopheline mosquitoes collected from October 1997 to November 1998 in southern Belize using 3 different collection techniques. *Anopheles* have been separated on the basis of collection method.

	Human	Cow	Pig	Dog	Cat
Indoor collections					
Anopheles vestitipennis	7.5	—	0.6	0.8	0.6
Anopheles darlingi	7.69	0	0	0	0
Outdoor collection					
An. vestitipennis	5.3	0.2	1.3	3.3	—
An. darlingi	5.2	0.8	0	0	0
Backpack aspiration					
An. vestitipennis	3.76	1.1	1.3	0.16	—
Anopheles albimanus	0.73	2.3	2.0	0.33	—
Anopheles punctimacula	—	1.5	7.2	—	—
Vehicle-mounted trap					
An. vestitipennis	2.0	2.2	0.2	0.4	—
An. albimanus	—	3.2	0.1	0.65	—
An. punctimacula	0.07	3.2	—	0.37	—

collections because there were no blood-engorged samples of this species collected on the surfaces of the houses. A comparison can be made, however, from the results of the backpack aspiration. Even though there were almost twice as many *An. vestitipennis* collected in this manner, a fairly large number of *An. albimanus* were also collected. Of these samples, 11% tested positive for human blood; this was considerably lower than the 44% of *An. vestitipennis* that contained human blood. The results from the vehicle-mounted trap were more dramatic in that, out of a sample of 103 blood-engorged specimens, no *An. albimanus* tested positive for human blood. The proportion feeding on cattle was very high (95%).

After conducting the feeding index analysis on the *An. vestitipennis* samples, the ratio of human blood to all other sources showed indices greater than 1 in all relationships except in the human:cow ratio collected from the car-top trap. After adjustment for the disparity in host size, however, this value also showed that the human host was preferred over cattle. The feeding index favoring dogs changed to show a preference for a canine host over humans after the adjustment. When looking at the broad picture, however, there appears to be a strong preference for *An. vestitipennis* to feed on humans over all other host sources.

The human blood indices for *An. vestitipennis* was consistent with the foraging ratios and the feeding indices. The high HBI of 75% for the indoor/outdoor collection shows a large proportion of this species contain human blood. Although the number of human hosts in the immediate vicinity was quite high, there were a large number of alternate host sources within flying distance. This indicates that *An. vestitipennis* has a strong tendency to feed on humans both indoors and outdoors when they are found near human habitations. A high HBI of 37.5% was also calculated for the backpack collection and the car-top collection.

In all instances, except for backpack aspiration collections, *An. albimanus* showed a preference for feeding on cattle over humans, pigs, and dogs. This agrees with Bangs (1999), who found this species to favor cattle and other domesticated animals over humans. *Anopheles punctimacula* also showed a strong tendency for feeding on domestic animals (i.e., cattle and pigs) over humans. The results from the foraging ratio analysis were very similar to the feeding indices. The samples obtained from the 3 collection methods showed that *An. vestitipennis* exhibits a strong preference for human blood. The strength of this association decreased as the area of collection was expanded outward away from an area of human habitation. This trend is understandable considering that, as the area of collection increases, the number of available nonhuman hosts also increases.

A field collection's degree of randomness is an important sampling issue. Collections conducted on the interior and exterior aspects of the house resulted in freshly blood-engorged females. These mosquitoes were resting in close proximity to the hosts on which they fed. The mosquitoes collected in the vehicle-mounted trap, however, contained blood meals that were several hours old and were a considerable distance from their host source. The larger sampling area includes a larger number of available hosts as well as a larger number of resting sites. These factors combine to make sampling the in-flight population a more random and unbiased (relative to host distributions) sampling method for blood-engorged females. The foraging ratio for *An. vestitipennis* collected with the vehicle-mounted trap indicated a feeding preference for both humans and cattle. The strength of the association was virtually the same for both human and cattle blood

(2.0 and 2.2, respectively). Again, it must be stressed that the foraging ratio makes no adjustment for the difference in body size between humans and cattle nor does it adjust for variables like host defensive behavior. This could lead to an unrealistic bias toward cattle that does not truly exist.

The foraging ratios for *An. albimanus* consistently demonstrated a feeding preference for nonhuman hosts. As with previous studies, *An. albimanus* seemed to prefer cattle and pigs to almost all other host species. In addition, the foraging ratios also demonstrated that there is an avoidance of human hosts. This type of feeding pattern is consistent with *An. albimanus*' crepuscular, exophagic feeding behavior. During times when this species is feeding, most humans are inside their homes eating or preparing for sleep. Therefore, the only hosts available to be fed upon are nonhuman. The same appears to hold true for *An. punctimacula,* which also showed a preference for cattle and pigs and an avoidance of human hosts. The association with pigs is weakened by the fact that the sample size on which the data are based is extremely small.

The foraging ratio for *An. darlingi* also is consistent with previously published data that indicate that this species is strongly anthropophagic. Samples taken from the interior and exterior aspects of the houses both indicate a strong feeding preference for human blood. This association, however, is weak due to the small numbers of *An. darlingi* collected. For this reason, further studies on the host feeding patterns of *An. darlingi* are required.

In summary, *An. albimanus* and *An. punctimacula* continually demonstrated a feeding preference for nonhuman hosts such as cattle and pigs. This zoophagic behavior along with a propensity for feeding outdoors makes for a weak vector association. Although *An. albimanus* has been shown to transmit malaria, it appears that environmental conditions that produce high vector population densities are required for transmission to occur within Belize.

Anopheles vestitipennis, on the other hand, showed a definite preference for humans in all 3 collection methods. This was further substantiated by the feeding indices, which showed a preference for humans over all other host types compared on an individual basis. This association was strengthened by factoring in differences in host body size. This anthropophagic behavior in conjunction with the fact that this species has demonstrated a natural infection with *P. falciparum* and *P. vivax* (VK210) by the sporozoite ELISA (Achee et al. 2000) and its behavior of readily entering a house to feed further incriminates this species as an important, if not the most important, vector of malaria in Belize.

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