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BLOODFEEDING BEHAVIOR OF *ANOPHELES SACHAROVI* IN TURKEY[1]

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ABSTRACT. The feeding habits of *Anopheles sacharovi* under natural conditions and in feeding rooms were investigated by use of the gel diffusion technique. Mosquitoes were collected from various villages of Çukurova and also from feeding rooms especially prepared for these experiments. Human, cow, sheep, chicken, horse, and donkey were used as hosts in these rooms. The results showed that *An. sacharovi* is a zoophilic species. The females preferred donkey when human, cow, sheep, chicken, and horse were equally available. Their preference changed to horse, cow, and sheep in the absence of donkey. The host preference index (HPI) was always smaller than 1 for humans in habitats offering a choice of hosts. The human blood index was high only in human dwellings. In other habitats numbers of mosquitoes feeding on animals were higher than on humans. Although the human blood index was low, *An. sacharovi* is the principal human malaria vector in Turkey partly because a significant proportion of those resting in human dwellings have fed upon the occupants, and partly because of the uneven distribution of human and animal hosts.

INTRODUCTION

Feeding behavior of vectors can be determined by analyzing blood meals. Identification of mosquito blood meals is an important criterion for malaria studies as the epidemiology of mosquito-borne diseases is intimately related to the natural feeding habits of mosquitoes.

Host preference is a choice between equally available sources of a blood meal. The human blood index (proportion of human bloodfed mosquitoes to total fed) represents the degree of mosquito–man contact (Garrett-Jones 1964). This index is important in establishing the relationship of known malaria vectors and their transmission potential to man. Although host preferences of mosquitoes are partly genetically determined, host-related and environmental factors also are important. For example, body temperature, humidity, smell, color, and size of the host are known as important factors affecting host preference. Factors affecting feeding habits and activities of vectors include habitats and the variety and numbers of available hosts (Royal et al. 1991).

Early precipitin tests of *Anopheles sacharovi* blood meals in Greece obtained positive human reactions in 61.5% of specimens taken from human dwellings and in 7.5% of those from animal shelters (Barber and Rice 1935). More recent studies in Greece reported positive human reactions in 38.5% of mosquitoes from human dwellings and 1.1% from animal shelters (Hadjinicolaou and Betzios 1973). Values of 26.4% from human dwellings and 9.4% from animal shelters were obtained in Iran (Edrissian et al. 1985). Human-positive reactions of *An. sacharovi* taken from a variety of shelters were 5.6% in Greece and 30.5% in Syria (Bruce-Chwatt et al. 1966). These findings suggest that feeding preferences of *An. sacharovi* vary from year to year and from place to place. There are no comparative figures from Turkey.

The purpose of this study was to obtain general information on feeding habits and host preferences in Çukurova of *An. sacharovi*, the principal malaria vector of Turkey.

MATERIALS AND METHODS

Blood-engorged females of *An. sacharovi* were collected from various villages of Çukurova from 1988 to 1990. The females were collected with mouth aspirators from human dwellings, animal shelters, abandoned places (unoccupied, ruined human dwellings and stables) around the villages and from feeding rooms that were especially designed for this study.

Feeding preference studies need special attention. According to the World Health Organization (1975), they may be undertaken with baited trap nets. Three types of feeding rooms were prepared (rooms 1, 2, and 3), each with different combinations of hosts. The feeding rooms were large (4 × 3 × 2. 5 m) with only 3 walls; one entire side was open. The hosts were placed on the floor about 0.5 m apart. Each night only one combination of the hosts was used. The hosts in room 1 were human, cow, sheep, and chicken; a horse was added to these hosts in room 2; and the horse was replaced with a donkey in room 3. Only one of each host was in each room. The hosts were left overnight in the feeding rooms.

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Mosquitoes from outside entered these rooms through the open side. Bloodfed wild females were collected with mouth aspirators at hourly intervals throughout the night, with not more than 10 min for each collection. Other mosquito species were also collected, but because our aim was *An. sacharovi,* only this species was investigated. Blood-host sources of mosquitoes were identified by using the gel diffusion technique.

Blood-engorged females collected from human dwellings, animal shelters, abandoned places, and feeding rooms were taken to the laboratory for testing. Blood drops from dissected abdomens were smeared onto Whatman no. 1 filter paper, air dried at room temperature, interleaved with nonabsorbent paper, and then stored in a refrigerator. Each smear was labeled. The agar gel diffusion technique described by Crans (1969) was used for the tests. Dried blood spots were eluted in 50 μl saline citrate for 30 min at room temperature. The eluted unknown blood sample was placed in the center well on an agar gel plate that had human, cattle, sheep, and equine antisera in the surrounding wells. Each blood meal was screened for several hosts in a single test.

Plates were covered and allowed to stand overnight at room temperature. Diffusion of the antigen (blood meal extract) and antisera took place in the agar gel and at the end of the reaction a distinct visible precipitin band occurred with one host antiserum. In multiple feeding situations, the reactions occurred between the blood smear and more than one host antiserum. Antisera of the hosts were prepared in our own laboratory (Köhler and Milstein 1975). To control the reliability of antisera for blood source identification, we fed *An. sacharovi* on various hosts. The known bloods were tested against antisera as above.

The feeding preference of mosquitoes on a given host can be evaluated by establishing the host preference index (HPI). The calculation of HPI is given below (World health Organization 1975):

$$\text{HPI} = \frac{\%\text{ mosquitoes with blood of host A}}{\%\text{ of host A among the total hosts}}.$$

If the index is 1, it indicates no preference, if it is higher than 1, it indicates preference for host A, and if it is lower than 1, it indicates a degree of avoidance (or less attractiveness) for the host, that is, negative preference. Comparison of the HPI to a series of hosts permits their relative attractiveness to be established for a particular species and set of environmental conditions.

The human blood index (HBI) was calculated in human dwellings and in other habitats. It was defined as the proportion of freshly fed *An. sacharovi* containing human blood.

RESULTS

The blood meals of 8,278 *An. sacharovi* females from special feeding rooms, and 9,522 females from human dwellings, animal shelters, and abandoned places were analyzed during the study (Table 1).

A total of 2,469 bloodfed *An. sacharovi* females were collected in room 1. The highest HPI was for cows (HPI = 2.98), followed by sheep (HPI = 1.18). Negative preference was shown for humans (HPI = 0.71) and chickens (HPI = 0.029).

A total of 5,305 bloodfed females were collected in room 2. Horse was the prefered host (HPI = 2.21), followed by cow (HPI = 2.04). Negative preference was found for human, sheep, and chicken.

In room 3, a total of 504 bloodfed females were collected. Donkey was the preferred host (HPI = 2.84), followed by cow (HPI = 2.09) and sheep (HPI = 1.01). There was negative preference for human and chicken blood.

The combined feeding room results showed that the preferred host of *An. sacharovi* was the donkey in the presence of other equally available hosts. In the absence of the donkey the preference order changed to horse, followed by cow and sheep. There was negative preference for human and chicken hosts, indicating that this is a zoophilic species that also feeds on humans. Multiple feeds were taken by 540 (7.0%) females in the 3 feeding rooms, which may be important in malaria epidemiology.

Cows, sheeps, goats, dogs, chickens, humans, and pigeons were found in the vicinity of human dwellings. A total of 1,421 bloodfed females were collected in human dwellings. Feeding preference was in the order of cow, human, sheep, horse, and chicken. Once again the most favored host was cow.

Cows, sheeps, horses, and chickens were found inside animal shelters and humans, dogs, and pigeons were found in the vicinity of these shelters. The horse was the least numerous of the hosts. A total of 6,734 bloodfed females were collected in this habitat. Feeding preferences were in the order of cow, sheep, human, horse, and chicken. Here, again the most favored host was cow, though a few mosquitoes had fed on humans.

A total of 1,367 bloodfed females was collected from abandoned places. The human blood index calculated from these places was 11.1%.

DISCUSSION AND CONCLUSION

In all habitats studied (feeding rooms, human dwellings, animal shelters, abandoned places), *An. sacharovi* was primarily a zoophilic species. The

Table 1. Numbers of female *Anopheles sacharovi* collected in special feeding rooms and different resting places in Turkey during 1988–90 with host preference indexes (HPI) determined by the gel diffusion test.

	Site								
	Room 1[1]		Room 2[2]		Room 3[3]		Human dwelling [no. (%)]	Animal shelters [no. (%)]	Abandoned places [no. (%)]
Host	No. (%)	HPI	No. (%)	HPI	No. (%)	HPI			
Human	296 (12.0)	0.71	457 (8.4)		40 (8.0)	0.55	353 (25.0)	199 (3.0)	76 (6.0)
Cow	1,226 (50.0)	2.98	1,805 (34.0)	0.5	151 (30.0)	2.09	747 (53.0)	4,767 (71.0)	1,143 (84.0)
Horse	—		1,962 (37.0)	2.04	—		48 (3.4)	183 (3.0)	3 (0.2)
Donkey	—		—	2.21	205 (41.0)	2.84	—	—	—
Sheep/goat	745 (30.2)	1.81	724 (14.0)	0.81	73 (15.0)	1.01	246 (17.3)	1,394 (21.0)	117 (7.0)
Chicken	36 (2.0)	0.029	22 (0.4)	0.009	6 (1.2)	0.02	2 (0.1)	82 (1.2)	7 (1.0)
Multiple feeding	166 (7.0)		345 (7.0)		29 (6.0)		23 (2.0)	71 (1.1)	9 (1.0)
Unknown	—		—		—		2 (0.1)	38 (1.0)	12 (1.0)
Total	2,469		5,305		504		1,421	6,734	1,367

[1] Feeding room with human, cow, sheep, and chicken as hosts.
[2] Feeding room with human, cow, sheep, chicken, and horse as hosts.
[3] Feeding room with human, cow, sheep, chicken, and donkey as hosts.

maximum preference was for horses or donkeys in feeding rooms and for cows in other habitats. The zoophilic tendency of *An. sacharovi* was also reported by other workers (Bruce-Chwatt et al. 1966, Hadjinicolaou and Betzios 1973, Edrissian et al. 1985). A negative preference was found for humans and chickens (HPI < 1).

In feeding rooms 2 and 3, the horse or donkey were the most attractive hosts. However, in other habitats, feeding on these hosts was very low. Horses and donkeys are fairly rare animals in Çukurova, but cows and sheeps are common. Thus, contact between mosquitoes and cows is much more frequent than between mosquitoes and horse or donkey. In nature the number and the distribution of hosts affects the preference index of mosquitoes (Schaefer and Steelman 1969, Edman 1971, Chandler et al. 1977). In this respect it should be mentioned that, during the harvest season, many thousands of itinerant workers sleep in the cotton fields of Çukurova, from which domestic animals are virtually absent (Ramsdale and Haas 1978). This ensures an abnormally high man : mosquito contact in this region at the height of the summer.

In human dwellings and abandoned places gel diffusion test results positive for cow were high (53 and 84%, respectively). This showed that the mosquitoes fed on cow outside before entering to rest in houses or abandoned places, indicating that *An. sacharovi* is an endophilic species. The endophilic character of *An. sacharovi* was also shown by Postiglione et al. (1973) in Turkey. In Italy, most female *An. sacharovi* that fed on animals were found resting in bedrooms; this also shows the endophilic character of the species (Russell et al. 1963). These results indicate that the feeding behavior of mosquitoes may not be determined by collecting information on resting places.

In all habitats and feeding rooms where bloodfed females were collected, it was estimated that *An. sacharovi* females showed a strong preference for animals. However, the percentage of human-fed anophelines in human dwellings was high (25.0%) compared with that in other habitats. These results are important from an epidemiological point of view, because this species is the principal human malaria vector in Turkey. In human dwellings this man–mosquito relationship should be enough for the vector to spread malaria. Although *An. sacharovi* is primarily a zoophilic species exhibiting a limited preference for human blood (HPI < 1), it still plays an

important role in transmission. Another important character in malaria transmission is multiple feeding. A single infected vector may spread the infection to more than one person in obtaining a full blood meal, which is of epidemiological importance (Klowden and Lea 1979). In our studies the incidence of multiple feeding in the feeding rooms was 6.3%. It was 17% for the same species in Greece (Boreham and Garrett-Jones 1973). Other studies have shown that mosquitoes seem sensitive to the activities of hosts while feeding (Wood and Wright 1968, Edman and Kale 1971, Klowden and Lea 1979). In our observations, females of *An. sacharovi* also preferred less active and more tolerant hosts to feed upon.

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