FEEDING BEHAVIOR PATTERNS OF ANOPHELINES FROM UTTAR PRADESH AND GUJARAT STATES OF INDIA

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ABSTRACT. Identification of host blood meals collected on filter paper in the field from fully fed female anophelines was carried out by a microdot-ELISA; field-caught mosquito species were simultaneously identified. The results indicate that the percentages of mixed feeds, i.e., mosquitoes feeding both on human and cattle hosts to complete a blood meal, was only 3–4%. Human and mixed feeds together accounted for nearly 10% of bloodmeals. Mosquitoes collected from human dwellings did not show a higher percentage of human feeding than cattle feeding. The human blood index was found to be related to the proportion of human and cattle population in an area.

INTRODUCTION

Feeding behavior of anopheline vectors is determined from the analysis of mosquito blood meals. The anthropophilic index (A.I.), defined as percentage of blood smears having human blood, is a very important parameter that gives the actual frequency of man-mosquito contact and, hence, measures the transmission probability. Reports on the A.I. of various anopheline species in India are scanty and show a wide variation. For example, for *Anopheles culicifacies* Giles, a major vector in India, indices ranging between 2 and 80% have been reported by Ramchandra Rao (1984). In the 3 southern states, however, the reported A.I. of this taxon is 2.5% in Madras (Russell and Rao 1942), 3.1% in Gujarat (Shalaby 1964) and 3.4% in Maharastra (Barber and Rice 1938). Surprisingly, the A.I. of this species in Sri Lanka varies from 23 to 66% (Garrett-Jones et al. 1980). In contrast, with *An. annularis* Van der Wulp, which is known to be zoophilic and prefers to rest in cattle sheds, the reported A.I. is as low as 1.3% in Orissa and 1.8% in Madhya Pradesh (Senior White et al. 1943). In several villages in Punjab, Pakistan, the reported A.I. for this species was only 0.8% (Reisen and Boreham 1979).

Another commonly encountered vector, *An. subpictus* Grassi, achieves a high seasonal density in India, but the reported A.I. for this taxon is only 3%. The published data on identification of blood meals from field caught mosquitoes are mostly from precipitin tests, a method of low sensitivity that probably gives lower estimates. Recently, these values have been seriously questioned by Collins et al. (1987) in a detailed study done in Orissa using an improved gel-diffusion technique. In our study we have employed a microdot-ELISA to analyze blood meals of wild-caught mosquitoes from Shahajahanpur and Ghaziabad in Uttar Pradesh (U.P.), and from Nadiad in Gujarat State. The sample size is fairly large and represents different ecosystems more than 1,000–1,500 km apart. We report here the observed blood meal preferences of 3 anopheline species, *An. subpictus*, *An. culicifacies* and *An. annularis*.

MATERIALS AND METHODS

Field collection sites: 1)Shahjahanpur district of U.P. state has 2 perennial rivers and 2 seasonal canals, and is a rich agricultural area. The major crops are wheat, rice, sugarcane, gram and moong lentils. A single rainy season extends from July to September. The annual rainfall averages 1,056 mm and the temperature varies from 10.0 to 44.5°C; the study population is 21,380. On an average, every third person keeps cattle. The slide positivity rate (SPR) and slide falciparum rate (SFR) were observed to be 75.8 and 11.4%, respectively, and the remainder was *Plasmodium vivax* in April–May 1986.

2)Ghaziabad district of U.P. is situated on the eastern bank of the River Yumuna and has an extensive irrigation canal network. It is a major wheat and sugarcane producing area; other crops like rice are also produced during the year. A door-to-door survey in Ghaziabad and actual counting gave the cattle:human ratio as 1:4, possibly due to an agricultural based economy. The SPR and SFR were found to be 27.2 and 15.2% in 1985, The rainfall averages 85 mm and temperature varies from 8.4 to 41.9°C.

3)Nadiad district of Gujarat is situated in the western part of India. It has an agrarian economy with dairying being one of the main occupations. The main crops are rice and cotton. This is an intensely irrigated area. The annual average rainfall is 1,673 mm and the temperature varies from 13.9 to 39.0°C. The SPR and SFR were 2.4 and 1.0%, respectively, in 1985.

In all 3 districts the major malaria vector is *An. culicifacies*, although other species caught were *An. subpictus*, *An. annularis* and *An. stephensi* Liston.

Collection of samples and processing: Indoor resting mosquitoes were collected with suction

tubes from either intradomestic or peridomestic sites of human dwellings or cattle sheds near human habitations, as well as from mixed dwellings. The species were identified and blood smears were collected on Whatman 3-mm filter paper discs and sent to the headquarters laboratory for identification.

The method used for identification of hosts from mosquito blood meals was by microdot-ELISA; the details of which have been published by Roy and Sharma (1987). Some mosquito bloodmeals, however, were identified as from neither human nor cattle sources and have been designated as "other" or noncattle host. The anthropophilic index (A.I.) was calculated by dividing the number of identified human fed mosquitoes by total number of mosquito bloodmeals analyzed and multiplying this figure by 100.

RESULTS

Host feeding pattern: A total of 1,678 mosquito blood meal smears were obtained from 15 villages of Shahjahanpur district. The pattern of host selection of wild-caught mosquitoes is reflected in Table 1. The major anophelines were *An. subpictus*, *An. culicifacies* and *An. annularis*. A few mosquito blood meals were positive with both the antihuman and anticow horseradish peroxidase conjugate, indicating mixed feeding. The human blood meal rate was 3.8% for *An. subpictus* and 3.9% for *An. culicifacies*, but if mixed blood meals are also considered, then the total percentage of blood meals from human sources worked out to 6.8% in *An. subpictus* and 5.6% in *An. culicifacies* (Table 1).

A sample of 339 mosquito blood meals was collected from the Ghaziabad area in 1988 (Table 2). *Anopheles subpictus* was not encountered here during the study period; *An. culicifacies* (336) and *An. annularis* (3) were tested. In *An. culicifacies*, the human blood feeding was 1.5% and mixed feeding 0.6%, the rest were cattle or others.

A total of 1,449 blood meals from Ghaziabad were tested (Table 3): *An. culicifacies* (966), *An. annularis* (93) and *An. subpictus* (390). In *An. culicifacies* the A.I. was 6.6% and mixed feeds were 4.3%; therefore, total human bites were 10.9% from the Dadri Primary Health Center. *Anopheles subpictus* had an A.I. of 0.77% and the mixed blood index was 2.6%. The highest total human feeding was 5.8%, the rest were cattle and others.

From Nadiad (Gujarat), 724 mosquito blood meals were collected (Table 4). The A.I. was 3.2% in *An. culicifacies*, 1.4% in *An. subpictus* and 3.7% in *An. annularis*; the remaining were from cattle or others. No test was carried out for mixed feeds. At Nadiad, where the Integrated Disease Vector Control program has been im-

Table 1. Variation in blood meal sources according to the type of resting place, Shahjahanpur, U.P., 1986.

Resting site	Species	No.	Blood meal source in % (no. of mosquitoes tested)			
			Human	Cattle	Human + cattle	Others
Human dwelling	*An. subpictus*	309	5.5 (17)	82.8 (255)	4.5 (14)	7.4 (23)
	An. culicifacies	158	5.7 (9)	75.3 (119)	1.2 (2)	17.7 (28)
	An. annularis	16	— —	43.7 (7)	— —	56.2 (9)
Cattle shed	*An. subpictus*	427	4.0 (17)	76.1 (325)	3.0 (13)	16.8 (72)
	An. culicifacies	170	4.1 (7)	82.3 (140)	2.3 (4)	11.2 (19)
	An. annularis	61	4.9 (3)	77.0 (47)	4.9 (3)	13.1 (8)
Mixed	*An. subpictus*	369	2.2 (8)	79.7 (294)	1.6 (6)	16.5 (61)
	An. culicifacies	137	5.1 (7)	78.2 (107)	2.9 (4)	13.8 (19)
	An. annularis	31	— —	74.2 (23)	— —	25.8 (8)
Total	*An. subpictus*	1,105	3.8 (42)	79.1 (874)	3.0 (33)	14.1 (156)
	An. culicifacies	465	3.9 (18)	78.3 (364)	1.7 (8)	16.1 (75)
	An. annularis	108	2.8 (3)	71.3 (77)	2.8 (3)	23.1 (25)

Table 2. Variation in blood meal source in anopheline according to type of dwelling, Ghadiabad, U.P., 1988 winter collection.

Resting site	Species	No.	Blood meal source in % (no. of mosquitoes tested)			
			Human	Cattle	Mixed	Others
Human dwelling*	*An. culicifacies*	107	1.9 (2)	79.4 (85)	0.9 (1)	17.7 (19)
Cattle shed	*An. culicifacies*	229	1.3 (3)	83.3 (190)	0.4 (1)	15.3 (35)
	An. annularis	3	—	66.6 (2)	—	33.3 (1)
Total	*An. culicifacies*	336	1.5 (5)	82.1 (275)	0.6 (2)	16.1 (54)
	An. annularis	3	—	66.6 (2)	—	33.3 (1)

* *An. annularis* was not found in human dwelling in Ghaziabad.

Table 3. Mosquito blood meal host identification in Ghaziabad, U.P., 1988 monsoon collection.

Primary health center	Species	Source of collection	No. of blood meals	Host identified				% hosts identified			
				Cattle	Human (H)	Mixed (M)	Others	H + M	Cattle	Others	Mixed
Dadri	*An. culicifacies*	House	368	309	24	16	19	10.9	84.0	5.2	4.3
		Cattle shed	207	185	12	6	4	8.6	89.3	1.9	2.8
Rajapur	*An. culicifacies*	Cattle	8	8					100		
Dhaulana	*An. culicifacies*	House	198	160	15	7	16	11.1	80.8	8.0	3.5
		Cattle shed	63	54	4	2	3	9.5	85.7	4.7	3.1
		Mixed dwelling	122	98	8	4	12	9.8	78.7	9.8	3.2
Dadri	*An. subpictus*	House	80	80	—	—	—	—	100	—	—
Rajapur	*An. subpictus*	House	8	7	—	—	1	—	87.5	12.5	—
Dhaulana	*An. subpictus*	Mixed dwelling	86	79	3	2	2	5.8	91.4	2.3	2.3
		House	120	117	—	1	2	0.8	97.5	1.7	.8
		Cattle shed	96	87	—	1	8	1.0	90.6	8.3	1.1
Dhaulana	*An. annularis*	House	93	90	—	—	3	—	96.8	3.2	—

Table 4. Studies on human and cattle blood meal source according to type of resting habitat, Nadiad Gujarat, 1985.

Resting site	Species	No. of MBMs*	% hosts identified	
			Human	Cattle and others
Cattle shed	*An. subpictus*	96	—	100
	An. culicifacies	108	—	100
	An. annularis	23	—	100
Human dwelling	*An. subpictus*	82	—	100
	An. culicifacies	112	3.6	96.4
	An. annularis	11	9.0	91.0
Mixed	*An. subpictus*	112	3.6	96.4
	An. culicifacies	106	5.7	94.3
	An. annularis	74	5.4	94.6
Total	*An. subpictus*	290	1.4	98.6
	An. culicifacies	326	3.2	96.8
	An. annularis	108	3.7	96.3

* Mosquito blood meals.

Table 5. Impact of human and cattle ratio on the feeding behavior *Anopheles culicifacies*.

Locality	Human/cattle ratio	Sample tested	Anthropophilic index
Shahjahanpur	3:1	38	2.6
Ghaziabad	4:1	32	6:3
Dadri	5:1	368	10.8
Dhaulan Primary Health Care Center	6:1	198	11.1

plemented for several years, the A.I. is low in vector populations of *An. culicifacies*. From cattle sheds, all hosts were detected to be cattle, whereas in human and mixed dwellings a low number of human hosts were detected. The results confirm that these anophelines are more zoophilic than anthropophilic.

Table 5 gives information on human and cattle ratios along with the anthropophilic index. It is evident that the A.I. is directly proportional to human and cattle ratios. At Shahjahanpur, the human:cattle ratio is low, whereas at Ghaziabad the ratio is high. This shows that with an increase in the number of cattle, the A.I. has decreased.

The data were also analyzed to find out whether the differences in feeding pattern, i.e., human or mixed feeding, had any relation to the type of resting place, i.e., human dwellings, cattle sheds or mixed dwellings. No correspondence was found between the feeding preferences and type of resting place.

DISCUSSION

Results of ELISA tests revealed that for the 3 vector species examined (in Shahjahanpur, U.P., and in Nadiad, Gujarat), the feeding preference on human hosts was only 3–4%, irrespective of their resting habitat. In contrast, in Ghaziabad, U.P., where no integrated vector control operation is in effect, the A.I. is somewhat higher, 6–7%. These results indicate the primary zoophagic nature of these anophelines. Further, among all the 3 anopheline species about 3–4% had mixed feeds. This finding is significant and should be taken into consideration in evaluating the human blood index and vectorial capacity. High seasonal variation is observed in the A.I. as shown in the results from Ghaziabad (Tables 2 and 3) villages, where cattle sheds and human dwellings are in close proximity, and in the monsoon, human dwellings become mixed dwellings. This explains the high proportion of mixed feed observed in many villages during the monsoon.

Anopheles culicifacies is the major vector of malaria in the Indian subcontinent. On an average, considering mixed bites, this vector shows an A.I. of 3–10%; the variation is related to the proportion of human and cattle population. Table 5 shows that an increase in cattle population decreases the A.I. significantly, presumably due to the primary zoophagic nature of *An. culicifacies*. These results are in main agreement with that reported by Afridi et al. (1939) in Delhi and by Jambulingam (1984) in Tamil Nadu and Joshi et al. (1988), who showed the predominantly zoophagic nature of sibling species A and B of *An. culicifacies* with a relatively higher degree of anthropophagy for species A with samples collected from Delhi and the states of U.P. and Bihar.

This report establishes that the microdot-ELISA, is useful for giving a quantitative evaluation of the A.I. and human blood index, including the mixed feeds. This ELISA-dot-blot-polyclonal procedure was recommended in an interlaboratory trial, organized by the World Health Organization (1987), as a simple visual test, which could be performed within a few hours.

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