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ANOPHELES FLUVIATILIS COMPLEX: HOST FEEDING PATTERNS OF SPECIES S, T, AND U

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ABSTRACT. The host feeding specificity of *Anopheles fluviatilis* sibling species S, T, and U was determined by analyzing blood meal source using countercurrent immunoelectrophoresis. A distinct difference in the feeding patterns was observed among these species. Species S was found to be predominantly anthropophagic with 91% of the population having fed on human blood. In contrast, species T and U were either exclusively or primarily zoophagic.

Anopheles fluviatilis James of the Myzomia Series is widely distributed in India although its role in malaria transmission varies from place to place. On the basis of variations in density, feeding behavior, and incrimination rates, the existence of distinct biological forms was suggested by several workers (Rao 1984). Recent study has shown that *An. fluviatilis* is a complex of 3 reproductively isolated species that can be identified cytologically (Subbarao et al. 1994). These have been provisionally designated as species S, T, and U. Because these species do not interbreed in nature, they are expected to show biological variations. A study was conducted, therefore, to examine the host feeding preferences of these species. Host feeding patterns of species S, T, and U, as observed in different districts of India, are reported in this paper.

Indoor resting *An. fluviatilis* were collected with suction tubes from cattle sheds and human dwellings in villages in the following areas during 1991–94: the districts of Dehradun, Hardwar, Allahabad, and in the terai and bhabar areas of Nainital in Uttar Pradesh State; the districts of Kheda and Bharuch in Gujarat State; and hilly forest areas of District Sundargarh in Orissa State.

Half-gravid females were utilized for identification of sibling species and blood meal source. Ovaries from each half-gravid female were removed and fixed in modified Carnoy's fixative (1:3 acetic acid: methanol). Blood from the gut of the same mosquito was smeared on Whatman No. 1 filter paper for blood meal assay. For each mosquito, its blood smear and the ovaries were given the same number.

Fixed ovaries were processed in 50% propionic acid and stained in 2% lacto-aceto orcein according to the method of Green and Hunt (1980) for making polytene chromosome preparations. Polytene chromosomes were examined under bright, field with a Zeiss Axioplan Universal microscope, and species S, T, and U were identified according to the diagnostic paracentric inversions (Subbarao et al. 1994). Blood meal samples were tested by countercurrent immunoelectrophoresis (CCIE; Bray et al. 1984). The proportions of females of each species found with human and a mixture of human and bovine blood were used to calculate the human blood index (HBI).

The results of blood meal analyses showing positive reactions to human and bovine antisera are given in Table 1. A small proportion of samples did not react with either of the sera tested. These mosquitoes might have taken blood from other animals for which the antisera tested were not specific. Because no variation was observed in the feeding preference of each species collected from human dwellings and cattle sheds in different villages of each district, the data were pooled.

In District Dehradun (Uttar Pradesh), collections made from Bapugram, where the human: cattle ratio was 11:1, revealed the presence of species T and U with a predominance of species T. The cytologically identified specimens of both species, when subjected to blood meal analysis, were found to be exclusively zoophagic. Likewise, in District Hardwar (Uttar Pradesh), in collections made from Ismilepur, where the human: cattle ratio was 4:1, species T and U were sympatric and totally zoophagic (Table 1).

In District Nainital (Uttar Pradesh), species T and U were found to be sympatric in Shanti Puri, Tilpuri, Matkota, and Kopa Lal Singh. In these villages, where the human: cattle ratio was 1:2.2, species U was exclusively zoophagic, whereas a few specimens of species T had fed on human blood. Thus species T was predominantly zoophagic with an HBI of 0.01 (Table 1). In the districts Allahabad (Uttar Pradesh), Kheda, and

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Table 1. Blood meal analysis of *Anopheles fluviatilis* sibling species.

District (state)	Year	Species	Total tested	Human +	Bovine +	Mix +	Non-reactive	Human blood index
Dehradun	1991–92	T	83	0	68	0	15	0
(Uttar Pradesh)		U	4	0	4	0	0	0
Hardwar	1991–92	T	11	0	11	0	0	0
(Uttar Pradesh)		U	71	0	69	0	2	0
Nainital	1991–94	T	567	1	538	4	24	0.01
(Uttar Pradesh)	1991–94	U	170	0	156	0	14	0
Allahabad (Uttar Pradesh)	1993	T	36	0	34	0	2	0
Kheda (Gujarat)	1993	T	29	0	29	0	0	0
Bharuch (Gujarat)	1994	T	38	0	38	0	0	0
Sundergarh	1992–94	S	156	135	4	7	10	0.91
(Orissa)	1992	T	3	0	1	2	0	—[1]
	1993	U	3	3	0	0	0	—[1]

[1] Not calculated due to low numbers.

Bharuch (Gujarat), only species T was found, and it was exclusively zoophagic (Table 1).

In District Sundargarh (Orissa), species S was the predominant species, comprising more than 98% of the total *An. fluviatilis* population in Manko and Birkera, situated in a hilly forest area, and Toda, in a mining area. Out of 156 specimens of species S tested, 113 were collected from Manko and Birkera and 43 from Toda. The human:cattle ratio in Manko and Birkera was 1.5:1, whereas in Toda it was 11.9:1. In all the study villages of this district, species S was predominantly anthropophagic, with 91% of specimens engorged on human blood (Table 1). Species T and U were encountered occasionally in very low numbers. Out of 3 specimens of species T, 2 were found with a mixture of human and bovine blood. Three specimens of species U were found with human blood in Toda, where the cattle population was very low. Therefore, it is difficult to comment on feeding preference of these species in this area.

It is evident from the data that a distinct difference in the host feeding preferences exists among the species of the *An. fluviatilis* complex. Variations in the host feeding patterns have been reported among the members of other species complexes, e.g., the *Anopheles gambiae* (White 1974) and the *Anopheles culicifacies* complexes (Joshi et al. 1988), which have an important bearing on malaria transmission in the areas of their distribution. In hilly forest areas of Bisra Primary Health Centre in District Sundargarh (Orissa), where species S is predominant and highly anthropophagic, malaria incidence is quite high, with an annual parasite incidence (API) of 157.4 and a predominance of falciparum malaria (Yadav et al. 1990). *Anopheles culicifacies* species B and C coexisting with *An. fluviatilis* in this area were found to be primarily zoophagic (Nanda et al., unpublished data). In contrast, in villages of the districts Dehradun and Hardwar (Uttar Pradesh), where species T and U are sympatric and zoophagic, malaria incidence is low, with the API ranging from 0 to 5.3 during 1991–92 (Sharma et al. 1995).

Host availability and preference are the 2 important factors that govern mosquito feeding patterns. A wide variation in the feeding preference of *An. fluviatilis sensu lato,* with an anthropophilic index ranging from 0 to 97%, has been reported by earlier workers (Rao 1984). In the present study, a marked difference was observed in the host feeding patterns of *An. fluviatilis* collected from different districts, which appear to be species specific. In spite of high human populations in the study villages of the districts Dehradun and Hardwar, species T and U were exclusively zoophagic. Similarly, species T and U examined from other districts, i.e., Nainital, Allahabad, Kheda, and Bharuch, were also zoophagic. In contrast, species S in the study villages of Manko and Birkera (human:cattle ratio 1.5:1) and Toda (human:cattle ratio 11.9:1) of District Sundargarh was found to be predominantly anthropophagic.

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