

HOST FEEDING PATTERNS OF *ANOPHELES CULICIFACIES* SPECIES A AND B

HEMA JOSHI, K. VASANTHA, SARALA K. SUBBARAO AND V. P. SHARMA

Malaria Research Centre (ICMR), 22-Sham Nath Marg, Delhi-110 054, India

ABSTRACT. Countercurrent immunoelectrophoresis was used to assay bloodmeals to determine the host specificity of *Anopheles culicifacies* species A and B, collected from areas in Delhi, Uttar Pradesh and Bihar. Results indicated the predominantly zoophagic nature of species A and B with a relatively higher degree of anthropophagy for species A. Further, the human blood index was found to be related to the proportion of human and cattle population in an area. This study is significant because, of the two species only species A was incriminated as the vector of malaria in these areas.

INTRODUCTION

Anopheles culicifacies sensu lato has a wide distribution extending from Iran in the west through India to Thailand in the east. It is also found in southern China and Nepal in the north and Sri Lanka in the south. It is an important vector of malaria in India and several neighboring countries. This species is predominantly zoophagic and becomes anthropophagic when the cattle population is low (Bhatia and Krishnan 1961, Jambulingam et al. 1984). Anthropophagic indices ranging between 2 and 80% have been reported for this species in India (Ramachandra Rao 1984). With discovery of 4 sibling species [A, B, C and D, in the taxon *An. culicifacies* (Green and Miles, 1980, Subbarao et al. 1983, Vasantha et al., unpublished data)], the question arose as to whether the differences observed in the rate of anthropophagy of this taxon are related to the biological variations existing among the sibling species. The differences observed in the feeding preferences in *An. maculipennis* Meigen populations from different regions in Europe was one of the factors which led to the concept of the possible existence of several biological species within a morphologically similar anopheline species in Europe (Hackett 1937). *Anopheles gambiae* sensu strictu and *An. arabiensis* Patton, two members of the *An. gambiae* complex were also reported to have differences in the anthropophagic index; *An. gambiae* s.s. was more anthropophagic (Coluzzi et al. 1975, Mollineaux and Gramiccia 1980). A preliminary study carried out in southern India on the feeding preferences of species A and B of the *An. culicifacies* complex revealed species A to be more anthropophagic than species B (Suguna et al. 1983). The sibling species of the *An. culicifacies* complex have specific distribution patterns (Subbarao 1984), variations in the seasonal prevalence (Subbarao et al. 1987), differential response to DDT (Subbarao et al. 1988a) and susceptibility to malaria parasite infection (Subbarao et al. 1988b). This study examines the blood meals of species A and B from Delhi,

and districts in Uttar Pradesh and Bihar, (areas in northern India). In northern India species A and B are sympatric (Subbarao et al. 1983, 1988c).

MATERIALS AND METHODS

Indoor resting *An. culicifacies* were collected with suction tubes from the following areas during 1985-87: Gopalpura, a periurban locality in Delhi; 18 villages in Ghaziabad, 9 in Bulandshahr and 8 in Jaunpur and Ballia districts in Uttar Pradesh and 3 villages in Saran district in Bihar. Ghaziabad and Bulandshahr are in western Uttar Pradesh whereas Ballia and Jaunpur are situated in the eastern part of Uttar Pradesh.

The procedures followed in the collection of *An. culicifacies* are similar to those described in Subbarao et al. (1988a). *Anopheles culicifacies* was collected during August 1985-June 1986 in Gopalpura, during January 1985-June 1987 in Ghaziabad, in July, August and November 1985 and March, July, August, September and November 1986 in Bulandshahr and in Jaunpur, Ballia and Saran in May, August and November 1985 and March 1986. *Anopheles culicifacies* collected from the field are brought to the laboratory for processing. After mild etherization half-gravid female mosquitoes having Christophers' stage III ovaries were separated. Only half-gravid females were used as it is only at this stage well developed polytene chromosomes are present which are suitable for reading the banding pattern. From each half-gravid mosquito, ovaries were removed and stored in a glass vial containing 1:3 glacial acetic acid and methanol fixative. From the same mosquito blood from the gut was smeared on Whatman No. 1 filter paper for blood meal assay and the rest of the mosquito was stored in a microfuge tube for sporozoite detection [results of which are published in Subbarao et al. (1988b)]. Each mosquito, its blood smear and the ovary were given the same number.

Ovaries were processed for polytene chromo-

Table 1. Bloodmeal analysis of *Anopheles culicifacies* sibling species.

Areas	Delhi		Ghaziabad		Bulandshahr		Jaunpur & Ballia		Saran	
	Species A	Species B	Species A	Species B	Species A	Species B	Species A	Species B	Species A	Species B
Hosts	Percentage positive									
Human	1.3	0.0	2.2	0.8	3.5	0.6	0.0	0.3	0.0	0.0
Bovine	98.5	100.0	94.0	97.0	90.0	96.0	100.0	99.0	0.0	100.0
Mixed	0.2	0.0	2.2	0.3	4.0	2.0	0.0	0.0	0.0	0.0
Not detected	0.6	0.0	1.6	1.5	2.5	1.8	0.0	0.6	0.0	0.0
Total samples tested	466	3	4345	382	833	493	6	332	0	75

Table 2. Monthly variations in human blood index (HBI) of species A and B of *Anopheles culicifacies* in district Ghaziabad.

Month ¹	Species A		Species B	
	No. tested	HBI	No. tested	HBI
January	1	0	0	0
February	0	0	0	0
March	0	0	0	0
April	130	0	1	0
May	514	0.01	5	0
June	390	0.04	5	0
July	572	0.11	15	0
August	607	0.08	33	0
September	315	0.06	56	0.02
October	519	0.03	107	0.02
November	437	0.04	100	0
December	19	0	0	0

¹ Samples collected in 1985 and 1986 were pooled.

some preparation following the procedure of Green and Hunt (1980). Polytene chromosomes were examined to identify the sibling species. Diagnostic inversion genotypes used for the identification of sibling species were: Species A - X +^a +^b 2 +^r +^h and species B - Xab 2g¹ +^h (Subbarao et al. 1983).

Bloodmeal samples were tested by counter current immunoelectrophoresis (CCIE) (Bray et al. 1984) and gel diffusion (Collins et al. 1986) techniques using agarose gels. Although both the techniques produced equally sensitive results in detecting the antigen up to 40 hours after the bloodmeal, CCIE was quicker as the electrophoretic run was completed within 25-30 minutes and precipitin arcs were sharper. Therefore, counter current immunoelectrophoresis was used for assaying the samples. Details of the technique are given below.

Barbitone buffer powder (0.5 M, pH 8.6) was obtained from Ms. Centron, India and 0.5 M stock solution was prepared. For gel buffer, stock solution was diluted in 1:12.5 ratio with distilled water and calcium lactate was added to obtain a final concentration of 0.5 gm/liter. For electrode (tank) buffer, stock solution was diluted in 1:5

ratio with distilled water and 0.375 gm calcium lactate per liter buffer was added.

Glass plates of 14 cm × 8.5 cm size coated with 0.2% agarose were layered with 0.9% agarose. The procedure used for coating and layering was that from Collins et al. (1986). Wells were punched 5 mm apart on the agarose gel with the help of a 1.5 mm diameter punch. Agarose pieces from the wells were removed with a syringe needle.

From blood smears 5 mm diameter discs were punched and soaked in the wells of a microtiter plate containing 50 µl of 0.65% normal saline. Smears were allowed to soak for 4 hours. Even when soaked for 2 hours satisfactory results were obtained. The antisera used in the assay were obtained from the Serologist and Chemical Examiner to the Government of India, Calcutta. The antisera were subjected to blind tests using known bloodmeal samples and various dilutions before they were used in the assays. On each agarose gel plate one blood sample of human, one of cow/buffalo and one of rabbit were assayed along with the test samples. On the human antisera plate, mosquitoes fed on cow and rabbit served as negative controls and on the bovine antisera plate, rabbit and human samples served as negative controls. Mosquitoes fed on human and cow blood served as positive controls on their respective plates. Antigen extracts were loaded on the cathodic wells while antisera were charged on anodic wells. Electrophoresis was performed at a constant voltage of 150 volts for 30 min. at room temperature. After the completion of the run, the glass plate was taken out from the electrophoretic chamber and immersed in 0.9% normal saline. After 10-15 min., results were read against a black background over an X-ray viewer.

RESULTS AND DISCUSSION

Results of *An. culicifacies* sibling species A and B specimens showing positive reactions to human and bovine antisera are given in Table 1. A small percentage of samples did not react

with either of the sera tested. These mosquitoes might have taken blood of other animals for which the antisera tested were not specific. Results showed that both species A and B were predominantly zoophagic as only a small proportion showed positivity against human antisera. Comparative chi-square values for the observed numbers of human, bovine and mixed positive antigens between species A and B were 8.8 ($P < 0.05$) in Ghaziabad and 16.27 ($P < 0.001$) in Bulandshahr. The human blood index (HBI) was calculated by adding the number of human as well as mixed (human + bovine) positive antigens. A statistical comparison of species A and B with regards to HBI in Ghaziabad ($\chi^2 = 9.5$, $P < 0.01$) and in Bulandshahr ($\chi^2 = 13.2$, $P < 0.001$) indicated that species A had a significantly higher HBI than species B. The chi-square values for HBI were calculated assuming that species A and B have equal preference to feed on human beings.

It can be seen from Table 1 that the proportions of species A and B vary in different areas. In Delhi and in villages of district Ghaziabad (U.P.), species A was the predominant species (>90%); in Bulandshahr (U.P.), its proportion was about 60% and in districts Jaunpur and Ballia (U.P.) and Saran (Bihar), species A was almost absent. Irrespective of such variations, species B remained less anthropophagic in all the areas. This strongly indicated that species B has less preference for human blood than species A. This difference was observed throughout the year (Table 2). Seasonal variations in the HBI of species A was noticed. It was low during winter (later part of November, December, January and February) and summer (April, May and June) months and significantly high during the monsoon and postmonsoon months (July, August and September). The HBI for species B was zero throughout the year except in September and October which however were not statistically significant.

In Gopalpura and districts Ghaziabad and Bulandshahr, malaria is endemic and species A

has been incriminated as the primary vector, whereas in Jaunpur, Ballia and Saran malaria is almost absent (Subbarao et al. 1988b, 1988c). Species A had a relatively high sporozoite rate in August (1.09%) and September (2.07%) in comparison to other months (Subbarao et al. 1988b). During the other months it was always less than one. The proportions of human blood feeding observed for species A and species B in this study are in accordance with the earlier reports for *An. culicifacies* sensu lato (summarized by Ramachandra Rao 1984).

Table 3 gives the data on the bloodmeal analysis of mosquitoes collected from different resting sites in district Ghaziabad. Comparative chi-square value of 7.9 ($P < 0.01$) indicated that the proportion positive against human and bovine antisera differed significantly in cattle sheds and human dwellings for the species A. The proportion of mixed positives was higher in cattle sheds than in human dwellings ($\chi^2 = 6.25$, $P < 0.02$). Presence of undigested blood from an earlier complete bloodmeal can be ruled out because the mosquitoes used were in half-gravid stage and CCIE is not sensitive beyond 40 hours of blood feeding. Hence, the most probable explanation for the presence of mixed positives is that the mosquitoes which are half fed on human blood go to cattle sheds to complete their bloodmeal on cattle. In all these villages, cattle sheds and human dwellings are situated in close proximity and during rainy season, most of the human dwellings become mixed dwellings. This may explain the high proportions of mixed positives observed in some villages and HBI in July, August and September.

The HBI of species A varied between the villages and was found related to the proportions of human and cattle populations (Table 4). Similar results have been reported for *An. culicifacies* in Delhi by Afridi et al. (1939) and in Rameswaram (Tamil Nadu) by Jambulingam et al. (1984). Since species A is the primary vector of malaria in areas where species A and B are sympatric (Subbarao et al. 1988 b), the predom-

Table 3. Bloodmeal analysis of *Anopheles culicifacies* species A and B collected from different resting sites in villages of district Ghaziabad

Resting sites	Species A				Species B			
	No. tested	Proportions with antigens of:			No. tested	Proportions with antigens of:		
		Human (H)	Bovine (B)	Mixed (H+B)		Human (H)	Bovine (B)	Mixed (H+B)
Cattle shed	181	0.02 (4) ¹	0.87 (158)	0.08 (15)	612	0.01 (1)	0.99 (161)	0.0 (0)
Human dwelling	142	0.04 (5)	0.93 (132)	0.01 (2)	116	0.0	0.99 (115)	0.0 (0)

¹ Figures in parentheses are observed numbers.

Table 4. Human blood indices of species A of *Anopheles culicifacies* as related to cattle population.

Villages	No. tested	Human blood index	Ratio
			Human:cattle
Ghaziabad			
Arthala	386	0.12	12:1
Chazarsi	716	0.02	1:1
Bhoopkheri	1790	0.01	1:1.5
Sirora	490	0.027	1:2
Surana	349	0.04	1:2
Delhi			
Gopalpura	463	0.015	1:1

inantly zoophagic behavior of species A can be exploited to reduce the transmission by increasing the animal population.

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