# OBSERVATIONS ON THE BLOOD FEEDING PATTERN OF THREE *ANOPHELES* SPECIES IN GUJARAT STATE, INDIA [1]

A. M. SHALABY [2]

INTRODUCTION. This article is a part of a general investigation which was initiated to study the host selection patterns of the primary and secondary vectors of malaria in Panchmahals district of Gujarat State in India. In another paper (Shalaby, 1969), the host preference of *Anopheles culicifacies* Giles, the chief malaria vector in the district is described. In the present article the blood feeding patterns of *Anopheles subpictus subpictus* Grassi, *Anopheles annularis* Wulp and *Anopheles fluviatilis* James which are found in variable abundance in the district have been investigated.

*A. subpictus subpictus* and *A. annularis* are anopheline species that are encountered in large numbers throughout the district of Panchmahals, the location and spray history of which have been given elsewhere (Shalaby, 1968 a&b). In 1960, both species were found resistant to DDT, the insecticide used in the spraying operations in the campaign for malaria eradication in the district. Shalaby (1968a) reported an $LC_{50}$ greater than 4 percent for *A. annularis* in the district. For reasons still unknown, these two species are considered as doubtful vectors of malaria in the Indian subcontinent. However, they have been found to harbor stages of the malaria parasite in nature (Covell, 1927, Iyengar, 1939, Russell *et al.*, 1939 and Russell and Rao, 1940). On the other hand, *A. fluviatilis*, a known and efficient vector of malaria, is considered an additional vector to *A. culicifacies* in Panchmahals district, if not the sole vector in other parts of Gujarat State. The susceptibility of this species to DDT has not been investigated as the numbers of the mosquitoes were insufficient for any tests to be made.

Reported here are the results of precipitin tests of blood meals of the three *Anopheles* species conducted at intervals during 1959–1962. The purpose was to obtain information which might prove useful to epidemiologists in their evaluation of the efficiency of those species as vectors of malaria in the area.

MATERIAL AND PROCEDURE. As has been stated elsewhere (Shalaby, 1969), the village conditions in Panchmahals district are similar to each other with animals predominating over people at an average ratio of 5:3. The dominant animal in the district is the cow which predominates over ox, ox over water buffalo, water buffalo over sheep and goat, sheep and goat over dog and dog over horse or donkey. Fowls are often found roosting in the bush around the animals. Pigs are generally rare. The majority of the village dwellings are in the form of mixed animal and human habitations where animal and man share the same room. Sheds with tethered animals alone are also found, but it is difficult to find dwellings occupied by humans only in these villages. The inhabitants normally sleep outside their dwellings in the summer season. At other seasons, the people and cattle occupy the same rooms at night.

Specific sampling sites within a number of villages representing the various talukas of the district were selected. There, collections of blood-engorged mosquitoes were made during the early hours of the morning. In order to have the outdoor

[1] This paper is based on work carried out during the period when the author was a staff member of the World Health Organization in India.

[2] Professor of Entomology, Department of Zoology, Faculty of Science, University of Alexandria, Alexandria, Egypt.

resting mosquitoes represented in this study, mosquitos were collected from three pit shelters, 1 x 1 meter wide and 1 meter deep, which were dug under a tree or in long grass near the vicinity of the breeding places at three of the villages in the district, namely, Motipura, Bhuval and Mandhra.

*A. subpictus subpictus* numbering 1217 were collected during July, August and September 1959 and February and March 1961; 763 and 454 blood meals were taken from specimens resting in mixed animal and human habitations and animal sheds, respectively. *A. annularis* totalling 484 were taken during September 1959 and February and March 1960; 236 and 248 blood meals were taken from specimens resting in mixed animal and human habitations and animal sheds, respectively. A total of 133 *A. fluviatilis* were collected during December 1961 and February and March 1962. The low density of that species inside dwellings made it difficult to find freshly fed mosquitos in large numbers. Only 44 females were collected from mixed animal and human habitations in Motipura village of Baria taluka. Freshly fed specimens were not found resting inside animal sheds. From the outdoor pit shelters at the villages of Motipura, Bhuval and Mandhra, 89 engorged females were taken. It is of interest to note that the outdoor resting *A. subpictus subpictus* and *A. annularis* collected from the pit shelters were few and were mostly gravid or half gravid, thus not suitable for precipitin tests.

The mosquitos collected were anesthetized and identified. The abdomens of the engorged specimens were separated from the head and thorax. The stomach with its contents of blood was pulled out with a needle and squashed on filter paper prepared for the purpose, using a clean glass rod. The filter papers generally used were of the circular type, 9 cm. in diameter; on each, 16 blood smears could be accommodated. Each filter paper was given a serial number and labelled for the species, locality from which the collection was made, type of resting place and date of collection. The blood smears were allowed to dry thoroughly before they were packed. In the majority of cases the blood smears were sent for a precipitin test soon after the collection was made. In the event of a delay of some days until a good-sized batch was collected, they were usually stored in a tightly closed jar and kept in a refrigerator before packing and dispatching to the testing laboratory.

Under arrangement with the World Health Organization, the blood smears taken during 1959 were sent for precipitin analyses to the Central Institute of Communicable Diseases at Delhi, India, while those taken during 1960, 1961 and 1962 were sent to the Lister Institute of Preventive Diseases, London.

RESULTS. Results of the various feedings of *A. subpictus subpictus* are shown in table 1. Of 763 feedings taken from the mixed animal and human habitations in nine villages of the district, 4.17 percent of the positive tests were taken from humans. The animal hosts were bovine and dog at blood ratios of 92.82 percent and 3.01 percent of the positive tests, respectively. Of 454 feedings taken from animal sheds at four villages of the district, three only were taken from human; 96.51 percent and 2.33 percent of the positive tests were the ratios for bovine and sheep or goat blood, respectively. One feeding showed mixed feedings on more than one host.

The results of the various feedings of *A. annularis* are shown in table 2. Of 236 feedings taken from mixed animal and human habitations at three villages of the district, only one was taken from human. The animal hosts were bovine at a blood ratio of 99.12 percent and sheep or goat at 0.44 percent of the positive tests. Of the 248 feedings taken from animal sheds at three villages of the district, 94.83 percent of the positive tests were taken from bovine and 3.75 percent from sheep or goat. Three females showed mixed feedings on more than one host.

The results of the various feedings of *A. fluviatilis* are shown in table 3. It will be seen from the table that the proportions

TABLE 1.—Identification of blood meals of *A. subpictus subpictus* from Panchmahals district.

| Locality | Date of collection | No. smears | No. giving positive results | percent positive for | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | human | bovine | dog | horse | sheep or goat | avian | Mixed animals |
| Collected from mixed animal and human habitations | | | | | | | | | | |
| Motipura (Baria taluka) | July '59 | 158 | 59 | 5.08(3) | 89.84(53) | 5.08(3) | .. | .. | .. | .. |
| Piplod (Baria taluka) | July '59 | 68 | 20 | 5.0(1) | 90.0(18) | 5.0(1) | .. | .. | .. | .. |
| Dohad (Dohad taluka) | Aug. '59 | 80 | 29 | 13.80(4) | 86.20(25) | .. | .. | .. | .. | .. |
| Omidpura (Limkheda tal.) | Aug. '59 | 64 | 30 | 6.66(2) | 73.34(22) | 20.0(6) | .. | .. | .. | .. |
| Baria (Baria taluka) | Aug. '59 | 50 | 24 | 4.17(1) | 91.66(22) | 4.17(1) | .. | .. | .. | .. |
| Prattapura (Limkheda tal.) | Sept. '59 | 49 | 27 | 7.40(2) | 92.60(25) | .. | .. | .. | . | .. |
| Motijari (Limkheda tal.) | Sept. '59 | 32 | 22 | 4.54(1) | 90.92(20) | 4.54(1) | .. | .. | .. | .. |
| Dewjini Serswani (Jhalod taluka) | Sept. '59 | 38 | 20 | .. | 100.0(20) | .. | .. | .. | .. | .. |
| Limkheda (Limkheda tal.) | Sept. '59 | 48 | 36 | 8.33(3) | 91.67(33) | .. | .. | .. | .. | .. |
| Motipura | Feb. '61 | 64 | 64 | 1.56(1) | 96.88(62) | 1.56(1) | .. | .. | .. | .. |
| (Baria taluka) | March '61 | 112 | 100 | .. | 100.0(100) | .. | .. | .. | .. | .. |
| Subtotal | | 763 | 431 | 4.17(18) | 92.82(400) | 3.01(13) | .. | .. | .. | .. |

TABLE 1.—*(Continued)*—Identification of blood meals of *A. subpictus subpictus* from Panchmahals district.

| Locality | Date of collection | No. smears | No. giving positive results | percent positive for | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | human | bovine | dog | horse | sheep or goat | avian | Mixed animals |
| | | | | Collected from animal sheds | | | | | | |
| Dewjini Serswani (Jhalod taluka) | July '59 | 32 | 20 | 5.0(1) | 90.0(18) | .. | .. | 5.0(1) | .. | .. |
| Motipura (Baria taluka) | Aug. '59 | 64 | 33 | .. | 90.91(30) | .. | .. | 9.09(3) | .. | .. |
| Dohad (Dohad taluka) | Sept. '59 | 48 | 25 | 4.0(1) | 96.0(24) | .. | .. | .. | .. | .. |
| Prattapura (Limkheda tal.) | Sept. '59 | 96 | 51 | 1.96(1) | 94.12(48) | .. | .. | 3.92(2) | .. | .. |
| Motipura | Feb. '61 | 130 | 130 | .. | 99.23(129) | .. | .. | 0.77(1) | .. | 1.19(1) |
| (Baria taluka) | March '61 | 84 | 84 | .. | 97.68(82) | .. | .. | 1.19(1) | .. | .. |
| Subtotal | | 454 | 343 | 0.87(3) | 96.51(331) | .. | .. | 2.33(8) | .. | 0.29(1) |
| TOTAL | | 1217 | 774 | 2.71(21) | 94.44(731) | 1.69(13) | .. | 1.03(8) | .. | 0.13(1) |

N.B.[1] Precipitin tests conducted during July, August and September 1959 were carried out at the Central Institute of Communicable Diseases, Delhi, India; the other tests were carried out at the Lister Institute, London.

[2] The figures in parentheses represent the number of blood smears positive for the respective tests.

TABLE 2.—Identification of blood meals of *A. annularis* from Panchmahals district.

| Locality | Date of collection | No. smears | No. giving positive results | percent positive for | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | human | bovine | dog | horse | sheep or goat | avian | Mixed animals |
| Collected from mixed animal and human habitations | | | | | | | | | | |
| Motijari (Baria taluka) | Sept. '59@ | 30 | 21 | 4.76(1) | 95.24(20) | .. | .. | .. | .. | .. |
| Prattapura (Limkheda tal.) | Feb. '60 | 64 | 64 | .. | 100.0(64) | .. | .. | .. | .. | .. |
| | March '60 | 80 | 80 | .. | 100.0(80) | .. | .. | .. | .. | .. |
| Motipura (Baria taluka) | March '60 | 62 | 62 | .. | 98.40(61) | .. | .. | 1.60(1) | .. | .. |
| Subtotal | | 236 | 227 | 0.44(1) | 99.12(225) | .. | .. | 0.44(1) | .. | .. |
| Collected from animal sheds | | | | | | | | | | |
| Dewjini Serswani (Jhalod taluka) | Sept. '59@ | 120 | 85 | .. | 94.11(80) | .. | .. | 5.89(5) | .. | .. |
| Prattapura (Limkheda tal.) | Feb. '60 | 80 | 80 | .. | 92.50(74) | .. | .. | 3.75(3) | .. | 3.75(3) |
| Piplod (Baria taluka) | March '60 | 48 | 48 | .. | 100.0(48) | .. | .. | .. | .. | .. |
| Subtotal | | 248 | 213 | .. | 94.83(202) | .. | .. | 3.75(8) | .. | 1.42(3) |
| TOTAL | | 484 | 440 | 0.23(1) | 97.04(427) | .. | .. | 2.05(9) | .. | 0.68(3) |

N.B. 1. @ Precipitin tests carried out at the Central Institute of Communicable Diseases, Delhi, India; other tests are carried out at the Lister Institute, London.

2. The figures in parentheses represent the numbers of blood smears positive for the respective tests.

TABLE 3.—Identification of blood meals of *A. fluviatilis* from Panchmahals district.

| Locality | Date of collection | No. collected | No. giving positive results | percent positive for | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | human | bovine | dog | horse | sheep or goat | avian | Mixed animals |
| Collected from mixed animal and human habitations | | | | | | | | | | |
| Motipura (Baria taluka) | Dec. '61 | 19 | 19 | .. | 42.10(8) | .. | .. | .. | .. | 57.9(11) |
| Motipura | Feb. '62 | 15 | 15 | .. | 40.0(6) | .. | .. | .. | .. | 60.0(9) |
| (Baria taluka) | March '62 | 10 | 10 | .. | 10.0(1) | .. | .. | .. | .. | 90.0(9) |
| Subtotal | | 44 | 44 | .. | 34.09(15) | .. | .. | .. | .. | 64.91(29) |
| Collected from pit-shelters out of doors | | | | | | | | | | |
| Motipura (Baria taluka) | Feb. '62 | 18 | 16 | .. | 12.5(2) | .. | .. | .. | .. | 87.5(14) |
| Mandhra | Feb. '62 | 10 | 10 | .. | .. | .. | .. | .. | .. | 100.0(10) |
| (Baria taluka) | March '62 | 33 | 33 | .. | 27.27(9) | .. | .. | .. | .. | 72.73(24) |
| Bhuval (Baria taluka) | March '62 | 28 | 27 | .. | 14.81(4) | .. | .. | .. | .. | 85.19(23) |
| Subtotal | | 89 | 86 | .. | 17.44(15) | .. | .. | .. | .. | 82.56(71) |
| TOTAL | | 133 | 130 | .. | 23.08(30) | .. | .. | .. | .. | 76.92(100) |

N.B. 1. Precipitin tests were carried out at the Lister Institute, London.
2. The figures in parentheses represent the numbers of blood smears positive for the respective tests.

of multiple feedings on more than one host are unusually high. Of 44 feedings taken from specimens resting in the mixed animal and human habitations and 89 feedings taken from the outdoor resting specimens, the proportions of mixed feedings were as high as 64.91 percent and 82.56 percent, respectively. The other feedings were all from the bovine host.

DISCUSSION. The overall objectives of this work were to determine 1) the human blood ratios of *A. subpictus subpictus, A. annularis* and *A. fluviatilis,* 2) the various hosts on which they feed and 3) the host preferences.

The results of precipitin tests presented herein clearly indicate that *A. subpictus subpictus* and *A. annularis* prefer animal to human blood. Of 1217 *A. subpictus subpictus* and 484 *A. annularis* captured from mixed animal and human habitations and animal sheds in Panchmahals district of Gujarat State, the ratios for animal blood were 97.29 percent for *A. subpictus subpictus* and 99.77 percent for *A. annularis* of the tests giving positive results. In the district where cow, ox, buffalo, sheep or goat, dog, horse or donkey and chicken are common animals, bovine was the principal host for the two species. Of the positive tests, the proportions for bovine blood were 94.44 percent and 97.04 percent for *A. subpictus subpictus* and *A. annularis,* respectively (Tables 1 and 2). The other animal hosts selected by *A. subpictus subpictus* were dog at a ratio of 1.69 percent and sheep or goat at 1.03 percent of the positive tests which are significantly low (Table 1). It is to be noted that the feedings on dog occurred in mixed animal and human habitations, while the feedings on sheep or goat occurred in animal sheds. Besides bovines the other animal hosts selected by *A. annularis* are sheep or goat only at a significantly low ratio of 2.05 percent of the positive tests (Table 2).

The role of *A. subpictus subpictus* in the transmission of malaria is not known in spite of its abundance in the district. It has been reported to enter dwellings for feeding at peaks of entry in the early morning and late evening (Shalaby, 1964). In Assam State in India, Ramsay *et al.,* (1936) reported a human blood ratio of 1.8 percent for this species. Afridi *et al.,* (1939), in parts of India where this species is found in large numbers reported a zero ratio for human blood. But Roy (1943) reported a human blood ratio of 25 percent which is considered to be high for an anopheline with a doubtful role in the transmission of malaria.

The results of precipitin tests presented in Table 1 show a human blood ratio of 2.71 percent of the positive tests. In the mixed animal and human habitations where the mosquitos had the opportunity to feed on either man or animal, a human blood ratio of 4.17 percent of the positive tests has been recorded. In Indonesia, results of precipitin tests of blood meals of *A. subpictus malayensis* and *A. subpictus subpictus* in 1955–1956 showed human blood ratios of 1.7 percent and 24 percent, respectively although *A. subpictus subpictus* is considered as an unimportant or unknown vector of malaria in Indonesia (Anonymous, 1959). On the other hand, Garrett-Jones (1964) reported a zero index for human blood in *A. subpictus subpictus* taken from areas under dieldrin spraying in Indonesia.

It is important to note that of 454 feedings taken in animal sheds, three mosquitos from three different villages were found to contain human blood (Table 1). It is obvious that these females must have fed elsewhere, although they came to rest in the animal shed. Anophelines that are found resting in a dwelling, therefore, have not necessarily obtained their blood meals from the occupants, as the mosquitos may feed in one place and rest in another.

*A. annularis* is another doubtful vector of malaria, although Bruce-Chwatt *et al.,* (1966) stated that this species might constitute a greater potential danger than *A. culicifacies* and *A. stephensi* in circumstances where the three vectors show comparable biting density and longevity. The results of precipitin tests presented in Table 2 show one feeding only on humans

in a mixed animal and human habitation, giving a human blood ratio of 0.23 percent of the positive tests. Afridi *et al.*, (1939) reported a zero ratio for human blood in parts of India where this species is found in abundance. Garrett-Jones (1964) reported a human blood index of 0.02 in this species from unsprayed areas in East Pakistan and India. Bruce-Chwatt *et al.*, (1966) reported a zero ratio for human blood and 99.3 percent for bovine in India. In Pakistan, they reported a ratio for human blood less than 1.0 percent. In Indonesia, it has been reported that results of precipitin tests of *A. annularis* during 1957–1958 recorded a human blood ratio of 0.6 percent (Anonymous, 1959). Much earlier, Walch (1932) reported a ratio of 10 percent for human blood which is considered a high ratio for an unimportant vector of malaria.

The results of precipitin tests of blood meals of *A. fluviatilis* are of much interest. Boyd (1949) indicated that this species in India showed a definite preference for human blood even in the presence of cattle. Covell and Harbhagwan (1939) reported a human blood ratio as high as 97.0 percent for this species in India. Jaswant Singh and Jacob (1944) reported a human blood ratio of 63.6 percent. Table 3, however, shows that of 133 records from mosquitos resting in mixed animal and human habitations and mosquitos resting out of doors, not one was positive for human blood. The proportions of feedings were bovine at a blood ratio of 23.08 percent and mixed feedings at 76.92 percent of the positive tests. In the mixed animal and human habitations, the proportions of feedings were bovine at 34.09 percent and mixed feeds at 64.91 percent of the positive tests (Table 3). Among the specimens resting in the pit shelters out of doors at the villages of Motipura, Bhuval and Mandhra, the proportions of feeding were bovine at 17.44 percent and mixed feeds at 82.56 percent of the positive tests (Table 3).

It was not possible to find freshly fed mosquitos resting in animal sheds, nor was it easy to find larger numbers of specimens in the mixed animal and human habitations due to the overall low density of *A. fluviatilis* in the district. This could be attributed to an exophilic tendency in the mosquito and/or to an irritant or repellent effect of the DDT on the mosquitos, where all dwellings in Panchmahals district have been treated with the insecticide. On the other hand, the recording of a zero ratio for human blood in a known vector of malaria like *A. fluviatilis* could, perhaps, be due to a change in the host selection behavior of the species produced by DDT pressure in an area like Panchmahals district with a history of over 12 years of intensive spraying.

At the same time, it is particularly noteworthy that while the proportions of mixed feedings in *A. subpictus subpictus* and *A. annularis* amounted to 0.13 percent and 0.68 percent, respectively (Tables 1 and 2), the proportion recorded in *A. fluviatilis* was as high as 76.92 percent. In most precipitin tests the proportion of mixed feedings does not exceed 5 percent (Anonymous, 1959). High proportions of mixed feedings, however, have been reported in *A. aquasalis* in Trinidad (Anonymous, 1959). Shalaby (1969) reported mixed feedings at a ratio of 24.31 percent in *A. culicifacies* collected from out of doors in Panchmahals district. Bruce-Chwatt *et al.* (1966) however, reported a 10 percent ratio of mixed feedings in *A. fluviatilis* from Iran, Iraq and Saudi Arabia. Nevertheless, the recording of such an unusually high ratio of mixed feeds in Panchmahals district during the period of the study is, it must be admitted, difficult to explain. It is therefore hoped that further investigations of the blood feeding pattern can be conducted on larger numbers of specimens with more seasons of sampling, so that some explanation can be offered for the host selection behavior of this species.

SUMMARY. The blood feeding patterns of *A. subpictus subpictus, A. annularis* and *A. fluviatilis* from Panchmahals district of Gujarat State in India were studied by analyses of host blood by precipitin test.

Of 1217 *A. subpictus subpictus* and 484 *A. annularis* collected from mixed animal and human habitations and animal sheds, animal feedings were most common with bovine being the principal host. Of the positive tests, the proportions of *A. subpictus subpictus* feedings were human 2.71 percent, bovine 94.44 percent, dog 1.69 percent, sheep or goat 1.03 percent and mixed feeds 0.13 percent. For *A. annularis* the proportions were human 0.23 percent, bovine 97.04 percent, sheep or goat 2.05 percent and mixed feeds 0.68 percent.

Of 133 *A. fluviatilis*, 44 collected from mixed animal and human habitations and 89 from out of doors, bovine hosts were the only ones identified. Mixed feedings at an inexplicably high ratio of 76.92 percent were recorded. No feedings on human were identified.

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