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DISTRIBUTION OF ANOPHELINES IN RELATION  
TO ALTITUDE IN NEPAL

by

C. P. Pant, WHO Entomologist, Amlckhganj, Nepal

G. D. Pradhan, Entomologist, Nepal Malaria Eradication Organization, Kathmandu

Y. Shogaki, WHO Entomologist, Kathmandu, Nepal

S. L. Shreshta, Entomologist, Nepal Malaria Eradication Organization, Amlckhganj

Distribution, abundance, longevity and ecology of anophelines is to a considerable extent dependent on the altitude and topography of a locality. Since much of the area of operations of the Nepal Malaria Eradication Organization falls within a mountainous region, an entomological altitude survey was carried out in localities representing various altitudes and topographical features during the summer and the autumn of 1960.

OBJECTIVES

The following were the broad objectives of the survey:

1. Determination of prevalence, distribution and density of anophelines at various altitudes up to 6000 feet above sea level.
2. Dissection of all the anophelines collected for sporozoites in the salivary glands.
3. Wherever possible, age-grouping of the most prevalent species and observation of any distinct differences in the proportions of parous and nulliparous mosquitos at different altitudes.
4. Observation of differences in the blood feeding and biting habits of the anophelines at various altitudes.
5. Formulation of the spraying policy guided by basic observations.

## METHODS

### Selection of the Area of Observations

It was considered that the valleys afforded better breeding places for the anophelines and hence at the higher altitudes (i.e., more than 4000 feet above sea level) valleys would be more important from the point of view of malaria transmission. According to the past records from these areas, malaria has been reported only from valleys. On the basis of altitude and topography, the following main types of areas were investigated:

#### A. High Valleys (see Table 1 and Map 1)

All the villages included in this group were more than 5000 feet and below 6000 feet above sea level. Slow running streams, rivulets and natural spring water provide ample breeding sites. There is also paddy cultivation in some of these villages and flooding of the fields during summer for transplanting seedling is quite common. The vegetation consists of a mixture of broad leafed and coniferous plants.

#### B. Medium Valleys

Valleys in the region of 4000 to 5000 feet are included in this group. Since Kathmandu Valley was the most easily accessible area, the majority of the work done in this area was in the above valley. Optimum conditions for anopheline breeding seemed to be provided in this area with diverse types of breeding grounds and plentiful water supplies throughout the year. Climate is also not very extreme. Ponds, rivulets, small mountain streams, natural spring water, artificial reservoirs, irrigation channels and flooded seedbeds of paddy were the main types of breeding ground.

#### C. Slopes

These are the cultivated hillsides between the altitudes of 3000 and 4000 feet above sea level. There are a large number of villages in this region and the vegetation is mostly of the broad leafed variety. Paddy, small millets, legumes and maize are the main crops grown in this area. There are plenty of sources of water in the form of naturally flowing small streams and irrigation ditches.

D. Low Valleys

These valleys are at the base of the hills and are surrounded by hills on all the sides. In altitude these range from 1500 feet to 3000 feet. Formed by the rivers, these valleys do not exceed 1/4 to 1/2 mile in width. Vegetation is of the broad leafed variety. Small rivulets and large rivers provide the breeding grounds. Cultivation of paddy along with a variety of other crops and fishing are main occupations of the inhabitants.

E. Pocket Valleys

At the confluence of small running streams, pocket valleys surrounded by hills are sometimes formed. These are covered with thick forests. Rice is normally cultivated in these valleys which are highly malarious.

Map 1 and Table 1 show the various areas surveyed. It may be appreciated that due to communication difficulties a much bigger sample could not be selected. Over the major part of the area mentioned in this survey, movement was possible only by foot.

Techniques

Most of the collections reported were made at dawn using a sucking tube. The mosquitos were brought to the field laboratory for identification and dissection. In a limited number of cases, age determination using the Detinova technique was also carried out. In some places the inhabitants did not co-operate and did not let the teams make mosquito collections in the human dwellings. Blood smear samples were taken from the stomachs of the freshly fed anophelines and forwarded to the Lister Institute, London, or Malaria Institute of India for precipitin tests.

The work was done by two teams. One consisted of the entomological section of the North Sector of the Nepal Malaria Eradication Organization, and the other of the Middle Sector of the same Organization. Each team worked under the constant supervision of the national and the WHO entomologists. Salivary gland dissections were examined by the national or WHO entomologist and the dissections for age-groupings were done by a WHO entomologist.

## RESULTS

During the course of the present survey, not a single specimen of A. minimus was collected. It may be mentioned here that in the foothills of Nepal A. minimus was incriminated as one of the vector species. It appears that the distribution of A. minimus is limited to the altitudes up to 2200 feet above sea level. However, during the course of the survey, this species was not encountered even within the altitude limit mentioned above. It appears that A. minimus is distributed only south of the Mahabharat range of mountains. North of this range A. minimus does not occur even at lower altitudes.

Tables 2 and 3 give the details of mosquito collection done by the two teams at various altitudes. At the higher altitudes, i.e., more than 4000 feet above sea level, A. hyrcanus was the most prevalent species followed by A. maculatus and A. annularis. A. fluviatilis was the next most prevalent species. Other species collected were A. subpictus, A. splendidus, A. culicifacies, A. lindesayi, A. tessellatus, A. gigas, and A. vagus.

In general the densities of anophelines were found to be higher during the post-monsoon period.

Table 4 shows the salivary gland dissections for different species during the pre- and post-monsoon periods. A total of 3271 mosquitos were dissected and no specimen was found to be infected with sporozoites.

Table 5 gives the results of age-grouping of some of the anophelines at various altitudes. Females with up to five dilatations in the pedicel of the ovarioles were found. Our observations showed that the period of gonotrophic cycle at the high altitudes of A. maculatus and A. fluviatilis was 72 hours upwards. There seem to be no significant differences between the proportion of parous females at the different altitudes. The proportion parous varies between 0.8 to 0.88 and the 95% confidence intervals expressed as percentage lie between the values given in the last column of Table 5.

Table 6 gives the results of blood precipitin tests. It is evident that these anophelines showed very low human blood ratio. The collections from the human dwellings were in general nil. Even use of pyrethrum spray catching did not yield

any anophelines from the human dwellings at the altitudes higher than 4000 feet above sea level. It would appear probable that the anophelines at the higher altitudes prefer animals to man for feeding. This may be one of the factors which could explain the absence of transmission of malaria at these altitudes in addition to adverse climatic conditions during at least part of the year.

#### SUMMARY AND CONCLUSIONS

1. No evidence of transmission was found in the high valleys (above 5600 feet altitude).
2. At the slopes at 3000 to 4000 feet above sea level, no evidence of transmission during the post- or pre-monsoon periods was obtained, but as a margin of safety the present practice of spraying up to 4000 feet above sea level may be continued.
3. In low valleys between 1500 and 3000 feet above sea level, high density of A. fluviatilis (up to 10 per man hour) during the post-monsoon period indicates that there is every likelihood of transmission especially during the post-monsoon period. Spraying is indicated.
4. In the medium valleys (such as the Kathmandu valley, 4300 to 4800 feet above sea level) there was no direct evidence of transmission during the pre- or post-monsoon periods. However, under certain favourable conditions, transmission may occur and a close watch should be maintained. Such favourable conditions might occur if there were a large number of infected immigrants and a high density of the potential vector species, A. fluviatilis, and A. culicifacies. Under the normal conditions spraying is not indicated.
5. At high altitudes, i.e., more than 4000 feet above sea level, only cattle sheds yielded large numbers of mosquitos. The blood precipitin tests made from stomach smears of A. annularis, A. fluviatilis, A. hyrcanus and A. maculatus indicated high preference to cattle at these altitudes.
6. These results should also be compared with parasitological findings at these altitudes and more definite recommendations should be made after considering both the parasitological and entomological evidence. In general the results of the parasitological survey made in the same area support the above observations. In the high valleys no positive cases of malaria were recorded.

TABLE 1

Name of Villages	Altitude Classification	Remarks
Bhamarkot, Samartar	Low Valley	1 500 to 3 000 feet above sea level
Dhunibesi, Thangri	Slopes	3 000 to 4 000 feet above sea level
Salimtar, Banepa Kathmandu Valley	Medium Valley	4 000 to 5 000 feet above sea level
Chitlang Palung	High Valley	5 000 to 6 000 feet above sea level
-	Pocket Valley	Below 1 500 feet above sea level. In this area work was conducted only by the North Sector team.

TABLE 2. MOSQUITO COLLECTIONS FROM DIFFERENT AREAS  
by Middle Sector Team

Species	Low Valley		Slopes		Medium Valley		High Valley		Grand Total
	Pre-monsoon Total %*	Post-monsoon Total %*	Pre-monsoon Total %*	Post-monsoon Total %*	Pre-monsoon Total %*	Post-monsoon Total %*	Pre-monsoon Total %*	Post-monsoon Total %*	
<u>A. hyrcanus</u>	0	7	0	1	481	225	49	424	1 187
<u>A. maculatus</u>	74	39	188	58	227	30	291	69	976
<u>A. annularis</u>	4	29	16	0	625	70	7	18	769
<u>A. fluviatilis</u>	48	58	25	0	39	35	42	104	351
<u>A. splendidus</u>	16	3	51	0	60	91	4	0	225
<u>A. subpictus</u>	0	0	0	0	22	9	0	4	35
<u>A. culicifacies</u>	0	15	0	0	11	0	0	0	26
<u>A. lindesayi</u>	0	0	0	0	1	0	4	2	7
<u>A. tessellatus</u>	0	0	0	0	0	2	0	0	2
<u>A. vagus</u>	0	0	0	0	0	1	0	0	1
									3 579

\* Percentage of grand total

TABLE 3. MOSQUITO COLLECTIONS FROM DIFFERENT AREAS  
by North Sector Team

Species	Pocket Valley		Low Valley		Slopes		Medium Valley		High Valley		Grand Total										
	Pre-monsoon Total	%*	Post-monsoon Total	%*	Pre-monsoon Total	%*	Post-monsoon Total	%*	Pre-monsoon Total	%*		Post-monsoon Total	%*								
<u>A. hyrcanus</u>	0	0	1	0.1	0	0	2	0.2	1	0.1	26	2.4	24	2.2	518	47.6	29	2.7	487	44.8	1 088
<u>A. annularis</u>	101	12.2	584	70.7	0	0	19	2.3	6	0.7	61	7.4	1	0.1	26	3.1	10	1.2	17	2.1	825
<u>A. tritaenatus</u>	13	1.7	278	35.9	12	1.6	41	5.3	104	13.5	138	17.9	95	12.3	45	5.8	17	2.2	30	3.9	773
<u>A. tritaenatus</u>	54	7.5	433	60.4	3	0.4	21	2.9	34	4.7	94	13.1	6	0.8	61	8.5	9	1.3	2	0.3	717
<u>A. subpictus</u>	8	2.8	3	1.1	19	6.7	244	86.2	2	0.7	6	2.1	0	0	0	0	0	0	1	0.4	283
<u>A. splendidus</u>	179	81.0	13	5.9	0	0	0	0	14	6.3	3	1.4	0	0	9	4.1	3	1.4	0	0	221
<u>A. culicifacies</u>	0	0	16	17.4	0	0	73	79.3	0	0	2	2.2	1	1.1	0	0	0	0	0	0	92
<u>A. thraex</u>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-	10	-	0	0	11
<u>A. tessellatus</u>	0	0	2	-	0	0	0	0	0	0	0	0	0	0	0	1	-	0	0	0	3
<u>A. bites</u>	0	0	0	0	0	0	0	0	0	0	0	0	1	-	0	0	0	0	0	0	1
																					4 014

\* Percentage of grand total

TABLE 4. SALIVARY GLAND DISSECTIONS AT VARIOUS ALTITUDES DURING THE PRE- AND POST-MONSOON PERIODS

Species	Low Valley				Slopes				Medium Valley				High Valley				Grand Total
	Pre-monsoon		Post-monsoon		Pre-monsoon		Post-monsoon		Pre-monsoon		Post-monsoon		Pre-monsoon		Post-monsoon		
	Dissected Positive	Dissected Positive	Dissected Positive	Dissected Positive	Dissected Positive	Dissected Positive	Dissected Positive	Dissected Positive	Dissected Positive	Dissected Positive	Dissected Positive	Dissected Positive	Dissected Positive	Dissected Positive	Dissected Positive	Dissected Positive	
<u>A. fluviatilis</u>	38	0	57	0	25	0	-	-	37	0	33	0	42	0	89	0	321
<u>A. maculatus</u>	68	0	36	0	188	0	58	0	225	0	18	0	284	0	42	0	919
<u>A. hyrcanus</u>	-	-	6	0	-	-	1	0	480	0	212	0	42	0	287	0	1 028
<u>A. annularis</u>	3	0	29	0	16	0	-	-	614	0	66	0	7	0	3	0	738
<u>A. culicifacies</u>	-	-	14	0	-	-	-	-	11	0	-	-	-	-	-	-	25
<u>A. splendidus</u>	15	0	-	-	50	0	-	-	60	0	88	0	4	0	-	-	217
<u>A. subpictus</u>	-	-	-	-	-	-	-	-	21	0	2	0	-	-	-	-	23
																	3 271

2 All data reported were collected by Middle Sector Team.

TABLE 5. RESULTS OF PHYSIOLOGICAL AGE-GROUPING  
OF ANOPHELINE<sup>3</sup> AT VARIOUS ALTITUDES<sup>2</sup>

Area	Species	Total dissected	Nulliparous	No. of dilatations					With sac	Total parous	Proportion parous	95% confidence interval (%) of proportion parous
				1	2	3	4	5				
High Valley	1. <u>A. maculatus</u>	117	25	48	28	9	2	1	8	94	0.80	68-85
	2. <u>A. fluvialis</u>	22	3	7	4	2	1	-	4	18	0.82	54-95
Medium Valley	1. <u>A. maculatus</u>	14	-	6	2	2	-	-	4	14		
	2. <u>A. hyrcanus</u>	66	10	33	13	7	-	-	3	56	0.85	71-92
	3. <u>A. culicifacies</u>	8	2	3	3	-	-	-	-	6		
Slopes	1. <u>A. maculatus</u>	26	3	13	7	2	-	-	1	23	0.88	67-98

<sup>2</sup> All the data reported here were collected by Middle Sector Team and the dissections were done by WHO Entomologist - Middle Sector.

TABLE 6. PRE- AND POST-MONSOON BLOOD PRECIPITIN TESTS AT DIFFERENT ALTITUDES<sup>a</sup>

Showing number positive for human blood

Elevation Species	Under 1 500 ft		1 501-3 000 ft		3 001-4 000 ft		4 001-5 000 ft									
	Pre-monsoon Tested Positive	Post-monsoon Tested Positive	Pre-monsoon Tested Positive	Post-monsoon Tested Positive	Pre-monsoon Tested Positive	Post-monsoon Tested Positive	Pre-monsoon Tested Positive	Post-monsoon Tested Positive								
<i>A. fluviatilis</i> AD <sup>b</sup>	39	2	55	4	34	5	113	9	3	0	54	6	11	2	3	1
" MD	5	1	45	1	9	2	124	8	0	0	4	0	1	0	6	1
" HD	1	0	20	2	0	0	24	4	0	0	5	2	0	0	0	0
<i>A. maculatus</i> AD	7	0	153	6	63	6	37	7	13	1	25	1	83	7	1	1
" MD	28	5	1	0	16	0	19	1	0	0	4	0	5	1	1	0
" HD	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0
<i>A. annularis</i> AD	30	7	153	4	13	0	41	1	58	6	100	4	9	4	1	0
" MD	0	0	0	0	1	0	13	1	0	0	20	0	3	0	4	0
" HD	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0
<i>A. culicifacies</i> AD	0	0	0	0	31	3	7	0	0	0	1	0	0	0	0	0
" MD	0	0	0	0	4	1	6	0	0	0	2	0	0	0	0	0
" HD	0	0	0	0	0	0	1	0	0	0	8	1	0	0	0	0
<i>A. splendens</i> AD	34	5	4	0	25	0	0	0	2	0	0	0	1	0	0	0
" MD	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
" HD	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
<i>A. subpictus</i> AD	2	0	2	0	0	0	0	0	1	1	0	0	0	0	0	0
" MD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>A. hyrcanus</i> AD	0	0	0	0	0	0	0	0	5	1	0	0	12	1	2	1
" MD	0	0	0	0	0	0	0	0	0	0	0	0	19	1	37	2

<sup>a</sup> All these data were collected by North Sector Team. Blood precipitin tests were carried out at the Malaria Institute of India, Delhi.

<sup>b</sup> AD - Animal dwelling  
MD - Mixed dwelling  
HD - Human dwelling

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FIG. 1  
SKETCH MAP OF NEPAL SHOWING LOCALITIES OF ALTITUDE SURVEY

