MALARIA TRANSMISSION IN HIGH MOUNTAIN VALLEYS OF WEST NEPAL INCLUDING FIRST RECORD OF ANOPHELES MACULATUS WILLMORI (JAMES) AS A THIRD VECTOR OF MALARIA*

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Introduction

Malaria transmission was proved entomologically in Nepal in 1955 for the first time (Peters et. al. 1955). The species incriminated then was *A. fluviatilis* from the valley of Chitwan. Later in the same valley it was proved that *A. minimus* is the primary vector. Brydon et. al. (1961) confirmed *A. minimus* as the primary vector and *A. fluviatilis* as the secondary vector in the forest belt of Terai and Inner Terai and in the hilly region *A. fluviatilis* as the only vector. Shrestha (1966) reconfirmed that the distribution of *A. minimus* is confined only in Inner Terai and the forest belt of Terai and of *A. fluviatilis* in the forest belt of Terai with occasional catches from plain cultivated Terai to mountain upto an elevation of 6,500 ft. above M.S.L. In 1965 *A. fluviatilis* was incriminated as a vector in a village at an elevation of 4,300 ft. M.S.L. in the district of Mugu. Same year (unpublished report by S.P. Pradhan) it was reported that some of the villages located in the high valleys of Mugu district had 8% to 13% parasite rates. Several entomological works had been carried out in order to incriminate the vector in the mountain valleys. Again in the year 1969 full fledged study was carried out in two valleys namely Khater and Gum in the District of Mugu. The study was started from May, 1969 and continued till July and September 24, 1969 in Khater and Gum respectively.

Description of the Areas

Both the Khater river valley and the Gum valley lie in the district of Mugu west in Nepal.

(a) **Khater river valley:** It is a valley of approximately 25 square miles with 15 miles as length. The altitude of the valley ranges from 3,800 ft. to 9,800 ft. M.S.L. There are about 65 villages in between the altitude of 3,800 ft. to 7,000 ft. Approximately 10,578 people are residing in those villages.

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* Submitted for Publication on 22/10/2026. (March 1970.)

(b) Gum valley: This is a valley made by the river Mugu Karnali of approximately 50 square miles with 25 miles as length. The altitude of the valley ranges from 3,500 ft. to 10,400 ft. M.S.L. There are about 100 villages inhabited by approximated 9,300 people in between the altitude of 3,800 ft. to 7,00 ft.

Thakuris and Khsatriyas are main groups of people in those valleys. Almost all the houses are two storeyed and made of stone walls. They are roofed with slate stone or hard dried cakes of made of clay and sand. Ground floors are always used as animal sheds and the upper floors are used for sleeping and cooking. As the walls are almost completely closed, it is too dark inside the room. In addition they use pine wood as their fire-wood which leaves heavy deposit of soot on the walls and ceilings.

The main crop of valleys are dry crops like barley and different varieties of millets (Italian, Indian and little millets) and wet crop like paddy. Paddy is grown only at the base upto an elevation of 6,800 ft M.S.L. The people start sowing Italian and Indian millets during June. By the middle of July they start planting paddy in lower terraces and little millet in the higher terraces where irrigation is not possible. Once the tilling of the lands is completed by the middle of July they move their animals to high altitude in order to save their crop from grazing. The shifting of animals is completed by the end of July and kept there until November when the harvesting is completed. During this period some of the animals are moved to the villages for 2 or 3 days only to help soiling their farms. This phenomenon is noted in most of the hill valleys of Nepal.

Limited movement of the people was noted. Few people were seen to be moving as porters. July, August and September are the busiest months in both the valleys. This is the period, when few villagers will move along with their cattles away from their villages. The major movement is between the villages and their hutments at the river beds during harvesting. During winter, the male folks move down to Terai for marketing and youngsters may move as far as Uttar Pradesh in India for earnings.

During the month of June and July the people have a habit of sleeping outside in the verandah.

Metereological Conditions

Tabulated below are temperature and rainfall recorded by the entomological teams at their camp sites at Sukadhik (4,300 ft M.S.L.) in Khater river valley and Bhambada (6,425 ft. M.S.L.) in Gum valley.

Table No. 1—Temperature and rainfall.		Temperature (°C)				No. of	
Place	Month	Average		Rainfall		rainy	
		Maximum	Minimum	Maximum	Minimum	in inches	days
Sukadhik	May	29.00	20.00	29.00	25.00		
	June	34.00	24.00	31.20	27.46		
	July	32.00	24.00	29.87	25.83		
Bhambada	May	28.00	10.00	22.12	17.98	1.58	9

June	28.00	20.00	25.85	22.15	3.24	15
July	25.50	21.00	23.61	21.51	10.33	20
August	27.00	20.00	24.56	21.09	9.17	18
September	25.00	13.50	22.30	18.09	8.40	18

Methods

In Khater valley seven villages lying at different altitudes were selected. Similarly in Gum valley nine villages were selected for the entomological work. From first week of August the villages Ruga and Luma were dropped out from the study as the bridge across the Karnali river was washed away by flood.

The names with their altitudes are given below. These villages were visited once in a week for morning collection. In addition the villages selected for night works were visited once in every fortnight for man-biting collections.

Khater river valley

1. Aam — 4,300 feet.
2. Riga — 4,300 feet.
3. Siyali bagar 4,200 feet.
4. Jeudi — 4,200 feet.
5. Hudi — 4,300 feet.
6. Sukadhik — 4,300 feet.
7. Kathiya — 4,300 feet.

Gum valley

1. Bhambada — 6,425 ft.
2. Shreenagar — 6,425 ft.
3. Chain — 6,500 ft.
4. Ima — 6,200 ft.
5. Lumsha — 6,100 ft.
6. Salim — 6,500 ft.
7. Balai — 6,500 ft.
8. Luma — 6,700 ft.
9. Ruga — 5,900 ft.

Entomological Techniques

In both places the following entomological techniques were applied for the study.

(a) Morning Indoor hand catches.

Usually this type of collections were done between 0600 hours to 0900 hours. But in Gum valley it is being done from 0400 hours to 0700 hours as it is observed that the anophelines were disturbed by smoke and human activity as early as 0600 hours. There aearly catches were carried out in the human and animal sheds in all the villages selected.

(b) Man biting catches — This is every fortnight, The local people were taken as the baits and collections were made continuously 2000 hours to 0500 hours.

(c) Larval catches as much as possible from all types of available breeding places.

(d) Dissection of salivary glands for examination of sporozites.

(e) Dissection of ovary to distinguish between parous and nulliparous.

(f) Susceptibility test of anophelines incriminated as vector.

In addition pyrethrum space spray catches and night resting collections were carried out in Khater river valley. Also the collections of anophelines from outdoor resting places were carried out in both the valleys. They are not reported in this paper.

Prior to entomological collections the malariometric surveys were carried out in both valleys and results are tabulated in table number 2. From the table it is revealed that the prevalence of malaria is higher in Khater river valley than in Gum valley and also the incidence is higher among children in both valleys.

Valleys	Age Group	Number Examined	Number Positive	Parasite Rate	Species of Plasmodium			
					F	V	M	MX
Khater river	0— 1 years	23	2	8.69	2	0	0	0
	2— 9 years	187	28	14.97	11	9	6	2
	10+ +years	440	28	6.36	9	16	3	0
	Total	650	58	8.90	22	25	9	2
Gum	0— 1 years	69	0	0.00	0	0	0	0
	2— 9 years	273	14	5.10	2	11	1	0
	10+ +years	545	16	2.90	5	10	0	1
	Total	887	30	3.40	7	21	1	1

Indoor hand catches were carried out during the early hours of the morning. As a result the following were observed.

Valleys	Months	Type of station	Man hours density of anopheline species (No. of females recorded during collection hrs)								
			flu	wil	mac	ann	spl	cul	sub	vag	lin
Khater river valley	May	Human	0.63 (35)	0	4.10 (227)	0.02 (1)	0.20 (11)	0	0	0	0.02 (1)
		Animal	2.06 (75)	0	9.71 (354)	0	0.81 (28)	0	0	0	0.16 (6)
		Total	1.19 (111)	0	6.29 (589)	0.01 (1)	0.41 (39)	0	0	0	0.07 (7)
	June	Human	1.06 (75)	0	4.77 (248)	0.05 (3)	0.28 (15)	0.02 (1)	1.06 (55)	0.02 (1)	0
		Animal	7.25 (216)	0	12.48 (372)	0.57 (17)	1.72 (51)	0	1.88 (56)	0.03 (1)	0
		Total	3.57 (291)	0	7.58 (620)	0.24 (20)	0.80 (66)	0.01 (1)	1.38 (111)	0.02 (2)	0
	July	Human	17.61 978	0	10.09 560	1.99 111	0.04 2	0.30 27	20.71 1149	0	0
		Animal	34.37 961	0	21.28 595	1.82 51	0.14 4	0.28 8	9.51 266	0.03 1	0
		Total	23.22	0	13.83	1.94	0.07	0.27	16.94	0.01	0

		1939	1155	162	6	25	1415	1	
May	Human	0	00	0	0	0	0	0	
	vally	0	0 0.11	0	0	0	0	0	0
			4						
June	Mixed	0	0 0	0	0	0	0	0	0
	Total	0	0 0	0.05	0	0	0	0	0
				(4)					
July	Human	0	0 0.07	0	0	0	0	0	0
	Animal	0	0 3.70	0	0	0	0	0	0
			(122)						
August	Total	0	0 1.67	0	0	0	0	0	0
			(125)						
	Human	0.03	0.14 0.07	0	0	0	0.01	0	0
Sept.	Animal	0.03	28.19 0	0.03	0	0	0.63	0	0
		(1)	(755)	(1)			(16)		
	Total	0.03	9.03 0.04	0.01	0	0	0.20	0	0
Sept.		(3)	(763) (4)	(1)			(17)		
	Human	9.98	0.03 0.08	0	0	0	0.02	0	0
		(466)	(11) (4)				(1)		
Sept.	Human	0	2.19 0	0.04	0	0	0	0	0
			(49)	(1)					
	Anjmal	0	69.82 0	3.45	0	0	0	0	0
Sept.			(81)	(4)					
	Total	0	5.52 0	0.21	0	0	0	0	0
			(130)	(5)					

Note:—flu=fluviatilis, wil=maculatus willmori, mac=maculatus maculatus, ann=annularis, lin=lindesayi, spl=splendidus, cul=cullifacies, sub=subpictus, vag=vagus.

The above table reveals that in Khater river valley *A. fluviatilis* is predominant in the month of July and majority of them were collected from animal sheds. During other two months *A. maculatus maculatus* were found to be predominant. It is to be noted that *A. maculatus willmori* were not collected resting inside premises during those three months, even though a few were collected from outdoor shelters. As already stated above outdoor collections and few others are not reported here.

In Gum valley *A. maculatus maculatus* was the only species recorded during the month of May and June. During the rest three months *A. maculatus willmori* was found to be predominant with few collections of the other species. Only 3 *A. fluviatilis* were recorded in the month of July from the village Lumsha. Mention may be made that in the month of August collection was not reported from animal sheds for the reason already stated above. This phenomenon probably forced the species to feed upon man during the month.

It is noteworthy here that the team leader noted the presence of *A. maculatus willmori* in a village named Chhapre lying at an altitude of 10,400 ft. above M.S.L. (nearby the Rara Lake).

Russel et. al. stated in their book "Practical Malariology" 1963 edition that *A. maculatus willmori* were found between 2,000 and 8,000 ft., *A. fluviatilis* between 1,000 ft. to 5,000 ft. and *A. maculatus maculatus* reached its upper limit at 4,000 ft. In Nepal the distribution of these species were recorded even at higher altitudes as described above.

Tabulated below is the man biting densities of anopheline species for both valleys. The collections were carried out upon local human baits continuously from 2000 hours to 0500 hours.

Table No. 4 - Monthwise man biting densities of anopheline species for both valleys.

Valleys	Months	Condition of human baits	Bait nights	Density of anopheline species in per man per night. (Number captured biting upon baits)				
				flu	will	mac	ann	sub.
Khater river valley	May	Int. dom.	3	0	0	0	0	0
		Peri. dom.	2	0	0	0.56 (1)	0	0
	June	Ext. dom.	6	0	0	0	0	0
		Peri. dom.	15	0	0	0.06	0	0
	July	Ext. dom.	3	0	0	0	0	0
		Int. dom.	3	14.00 (42)	0	3.66 (11)	1.33 (4)	0.66 (2)
Gum valley		Peri. dom.	15	2.60 (39)		3.73 (56)	0	0
	May	Peri. dom.	12	0		0	0	0
	June	Peri. dom.	21	0		0	0	0
	July	Peri. dom.	24	0		0.41 (10)	0	0
	August	Peri. dom.	34	0		8.88 (302)	0	0
	Sept.	Peri. dom.	25	0		0.32 (8)	0	0

Note:- Int= Intra, Ext.= Extra, and Dom= Domiciliary.

From the table above it appears that *A. fluviatilis* is highly endophagic in Khater river valley. The species was recorded biting only in the month of July and the density per man night was 14 under intradomiciliary condition and 2.60 under peridomiciliary condition. In the same month *A. maculatus maculatus* was found biting upon human with the density of 3.66 to 3.73 whereas in Gum valley the only species collected biting man was *A. maculatus willmori* with the highest density in August, the month when there are no animals in the village. Although three odd *A. fluviatilis* were collected resting inside premises (Table No. 3) none were recorded biting.

The dissection of salivary glands for the examination of sporozoites were carried out in both the valleys. Beside the species given below in table No. 5, a few of the other species were also dissected. As the numbers were too low they are not included here.

Table No. 5:— Monthwise summary of salivary glands of *A. flaviatilis*, *A. maculatus willmori* and *A. maculatus maculatus* with their results for both the valleys.

Valleys	Month	<i>A. flaviatilis</i>			<i>A. maculatus willmori</i>			<i>A. maculatus maculatus</i>		
		No. diss	No. posi	Sp. rate	No. diss	No. posi	Sp. rate	No. diss	No. posi	Sp. rate
Khater river valley	May	138	0	0				529	0	0
	June	344	0	0				623	0	0
	July	2052	3	0.15				983	0	0
	Total	2540	3	0.11				2129	0	0
Gum valley	May							4	0	0
	June				0	0	0	15	0	0
	July	3	0	0	598	0	0	3	0	0
	August				578	3	0.52	39	0	0
	September				107	1	0.93	0	0	0
	Total	3	0	0	1183	4	0.34	61	0	0

Note:— diss=dissected, posi=positive, Sp=sporozoite.

The table above reveals that *A. flaviatilis* is the vector responsible for the transmission of the disease in the Khater river valley by incrimination of the species in the month of July. The positive *A. flaviatilis* were collected from the villages Aam and Kathiya. This species have been incriminated by finding sporozoites in one *A. flaviatilis* first time in same valley in September 1965. During the same period adequate sample of *A. maculatus maculatus* of total collections were dissected and none were found to have sporozoites in their glands. In other valley of Gum *A. maculatus willmori* was incriminated in August and early September from the villages Bhambada, Chain and Shreenagar. Though a few *A. maculatus maculatus* were dissected none were found to have sporozoites.

Dissection of ovary to distinguish between parous and nulliparous females by Detionva method were made on three species of anophelines found in those valleys. From the proportion parous the value for "p" (survival of mosquitoes through one day) were calculated and given in table No. 6. The average temperature and humidity recorded in the valleys indicated that the gonotrophic cycle may be of 72 hours and accordingly the value of "p" is calculated. From the table it is revealed that among the wild caught population of *A. flaviatilis* in Khater river valley about 76% to 80% are surviving through one day. Similarly 70% to 72% of *A. maculatus willmori* are surviving through one day in nature in Gum valley.

Table No. 6:— Monthwise summary of ovary dissection of *A. flaviatilis*, *A. maculatus willmori* and *A. maculatus maculatus* with their results for both the valleys.

Valleys	Month	A. fluviatilis				A. maculatus will				A. maculatus mac			
		No. diss	No. par.	Prop	p	No. diss	No. par.	Prop	p	No. diss	No. par.	Prop	p
Khater river valley	May	66	28	0.42	762								
	June	116	59	0.51	802								
Gum valley	May									4	2		
	June									12	6	0.50	789
	July	3	1		202	71		0.035	708	0	0	0	0
	August	0	0	0	364	136		0.37	721	31	10	0.32	687
	Sept	0	0	0	17	33		0.38	728	0	0	0	0

Note:- Par.=parous, prop.=proportion.

Two complete series of susceptibility test, one each with *A. fluviatilis* from Khater river valley and *A. maculatus willmori* from Gum valley were carried out against DDT with one hour as an exposure period. The W. H. O. test kit and instructions were completely followed. The results obtained from the tests were tabulated in table No. 7. From the results it may be said that both the species are susceptible. However 3 out of 100 *A. fluviatilis* and 1 out of 107 *A. maculatus willmori* were found to survive after 24 hours holding period. To explain this some more detailed work is needed.

Table No. 7:- Results of Susceptibility test of *A. fluviatilis* (Khater river valley) and *A. maculatus willmori* (Gum valley) with D. D. T.

Insecticide		A. fluviatilis			A. maculatus willmori		
Concentration	No.	No.	Mortality	%	No. Tested	No.	Mortality %
	tested	dead				dead	
4.00 %	100	97	97		107	106	99.06
2.00 %	100	84	84		108	97	89.81
1.00 %	100	56	56		131	67	51.14
0.50 %	100	20	20		132	32	24.24
0,25 %	100	5	5		109	7	6.42
Control	100	0	0		107	0	0
LC50		0.92 %				0.86 %	
(Graphical)							

Summary:

Two valleys (Khater river valley and Gum valley) were selected for the study of Malaria transmission in hilly region. Along with entomological observations, malariometric surveys were carried out and parasite rate found to be 8.90 % for Khater river and 3.40 % for Gum valley. The entomological observations recorded *A. fluviatilis*, *A. maculatus maculatus*, *A. maculatus willmori*, *A. annularis*, *A. splendidus*, *A. vagus*, *A. culicifacies*, and *A. lindesayi* from Khater river valley. The predominant species was *A. fluviatilis* followed by *A. maculatus maculatus*. The per man hours density of *A. fluviatilis* was 17.61 for human sheds and 34.37 for animal sheds and the density per man per night was 2.60 to 14.00.

during July. In the same month 3 *A. fluviatilis* were found to have sporozoites in their glands. From Gum valley only five species of Anophelines-*A. fluviatilis*, *A. maculatus willmori*, *A. maculatus maculatus*, *A. annularis* and *A. subpictus* were recorded. The predominant species was *A. maculatus willmori* having per man hours density of 9.98 for human sheds in August and 69.82 from animal sheds in September and the density per man per night was 8.88 in August. This species was incriminated by finding sporozoites in 4 specimens (3 in August and 1 in first week of September).

The presence of *A. maculatus willmori* was noted upto an elevation of 10,400 feet above M.S.L.

The susceptibility tests with *A. fluviatilis* from Khater river valley and *A. maculatus willmori* from Gum valley against DDT found both of them were susceptible. Few survivors at 4.00% DDT concentration were noted.

Conclusion

From the result and discussion given above it may be concluded that the transmission of malaria in the valleys of Khater river and Gum of district Mugu is due to *A. fluviatilis* and *A. maculatus willmori* respectively.

Acknowledgement

The authors wish to thank Mr. A. Stephen, Chief Malaria Advisor, US/AID for providing US/AID STOL for transporting teams from Nepalgunj to Jumla. Thanks are also due to other team members without whose endeavour the study would not have been successful. Special thanks goes to Mr. S.P. Pradhan, Assistant Zonal Head of West Zone for his suggestions and help rendered throughout the study.

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