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MALARIA IN NEPAL: OBSERVATIONS DURING A SHORT-TERM ENTOMOLOGICAL CONSULTANCY AUGUST 19 - SEPTEMBER 12, 1986

by

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EXECUTIVE SUMMARY

At the request of the USAID/Kathmandu mission and the Nepal Malaria Eradication Program, I was sent to Nepal during August and September 1986 with the following objectives: (1) to provide entomological advice and assistance during a NMEO survey of the malaria situation in Kanchanpur District, Far West Region, designed to incriminate new anopheline vector species; (2) to review NMEO proposals on vector bionomics in the Central and Far West Regions; and (3) to assess training needs for NMEO personnel. In addition, NMEO staff requested input on Japanese encephalitis virus (JEV) surveillance and control.

Findings and recommendations: An epidemiological assessment of 61 recently detected malaria cases in Kanchanpur District by the WHO Malaria Advisor and NMEO staff using a patient interview approach indicated that only 7% could be attributed to indigenous transmission. Eight villages in agricultural, transitional and forested ecological settings (including two unsprayed villages) were surveyed entomologically using indoor and outdoor resting and all night human and bovid bait sampling methods.

The reputed vector species in the Far West, *An. fluviatilis*, was rare in outdoor and bovid bait catches and was not collected resting indoors or biting man. A secondary vector species incriminated previously in the Terai, *An. annularis*, was collected infrequently while biting bovid bait and could not be collected resting indoors or biting man. The incriminated forest vector species, *An. maculatus*, was collected resting outdoors and biting bovid baits in the transitional and forested zones and was never collected resting indoors. The most abundant anopheline collected in houses and biting man was *An. culicifacies*, an important vector in India which has yet to be incriminated in Nepal. *An. culicifacies* and *An. annularis* were found to be resistant to DDT and exhibited low-level survival to malathion.

The persistence of low-level malaria transmission in the absence of the reputed malaria vectors will require long-term longitudinal research to relate anopheline vectorial capacity to malaria transmission. Research should evaluate the impact of the house spraying program on anopheline vectorial capacity to malaria transmission. Research should evaluate the impact of the house spraying program on anopheline ecology and malaria incidence.

Submitted proposals were timely and address many current research needs of the residual house spraying program. Guidelines for revision were discussed with NMEO and USAID/Kathmandu staff. The proposed research is necessary to incriminate anopheline vectors and plan control strategies.

The NMEO is proposing to become a vector-borne disease control program. Expanded responsibility for arboviral and filariasis surveillance and control will require an increase in

NMEO staff, upgrading of the Hetauda Research and Training Centre to include an arboviral diagnostic laboratory, and expanded training of mid- and upper-level staff by extramural personnel provided through the Vector Biology & Control Project. USAID is urged to support the construction of the new research and training facility at Hetauda.

Clinically diagnosed JEV cases were observed at Bheri Zonal Hospital, and emergency culicine control measures were discussed with the NMEO staff at the West Regional Headquarters in Nepalganj. Every effort should be made to provide laboratory confirmation of suspect JEV cases to define the scope of the JEV problem. NMEO should consider an animal (duck or piglet) sentinel program to detect the onset of transmission. The focal malathion ULV spray program in response to clinical case detection is anticipated to have little impact on JEV transmission. Emergency control effectiveness should be evaluated and most likely replaced with a culicine mosquito surveillance and control program using an integrated pest management approach emphasizing community participation in permanent source reduction.

I. INTRODUCTION

Nepal currently is undergoing a nationwide resurgence of malaria with the total cases detected annually increasing from 16,087 in 1981 to 42,321 in 1985.[2] Of considerable interest has been the >10 fold increase in the number of P. falciparum cases from 733 in 1981 (5% of total cases) to 7,581 in 1985 (18% of total cases). The most marked rise in P. falciparum cases occurred in the newly formed Far West Region, where an ongoing epidemic has contributed 73% of the total P. falciparum cases. Of the four districts in the Far West, case incidence has increased most markedly in Kanchanpur District (2.2/1000 in 1981 to 39.3/1000 in 1985).[3] Cases of both P. falciparum and P. vivax have been clumped seasonally, with maximal prevalence occurring during the June-September monsoon period. In Kanchanpur District, during 1985 two rounds of DDT were sprayed at the rate of 1.5 g/m^2 during 45-day periods beginning in April and August.[4] Spraying was focal and targeted only those villages with the highest malaria prevalence based on a stratified malaria control strategy (Table 1).[5] Less than 30% of the population of both districts was protected by residual house spraying. Mosquito control in sprayed villages must be considered a failure, since the malaria incidence was often greater in sprayed villages than in unsprayed villages.[5] Although there is considerable movement of migrant workers from India into Nepal, the Nepal Malaria Eradication Organization (NMEO) classified more than 85% of the malaria cases as attributable to indigenous transmission.[5]

The deteriorating malaria situation in the Far West was attributed to several interacting factors: (1) refractoriness to spraying by the anopheline mosquitoes, (2) population movements to and from India where extensive malaria epidemics have been reported, (3) inadequate case surveillance and treatment, (4) extraordinarily high precipitation during the monsoon seasons of 1984 and 1985, and (5) ecological changes associated with large-scale deforestation and population growth.[4]

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[2]Joint HMG/WHO/USAID/ODA(UK) external situation analysis team. March 1986. Report of an analysis of NMEO's activities for 1984 and 1985, 67 pp.

[3]Pradhan, S. P. January 1986. Malaria situation in the Far West. Field Trip Rep. 1, 8 pp.

[4]Nepal Malaria Eradication Organization. March 1986. Annual Evaluation Report, 1985. 73 pp.

[5]Pradhan, S. P. April 1985. Malaria situation in two different belts of Kailali and Kanchanpur Districts. Trip Rep. 3 pp.

Table 1. Number of Persons (% of total) Protected by DDT Spray in Two Far West Districts During 1986[5]

	Kailali	Kanchanpur
Round 1	39,569(11)	21,451(10)
Round 2	21,546(8)	63,778(30)
Total population	277,837	213,330

In response to the malaria situation in the Far West, the NMEO has proposed a year-long entomological investigation entitled, "Bionomics and role of transmission of anophelines in district Kanchanpur of Nepal." To provide background information necessary for implementing the proposed research, the NMEO headquarters staff planned a field trip to Kanchanpur District during August and September 1986 to (1) incriminate vectors other than An. fluviatilis, (2) evaluate anopheline susceptibility to insecticides, (3) verify the occurrence of chloroquine resistance, and (4) classify the origin of infection in cases detected during January-July 1986.

In July 1986, USAID/Kathmandu requested VBC participation in planning ongoing and proposed research (Ref: Kathmandu 04458) and a vector biologist to accompany the NMEO team to the Far West (Ref: Kathmandu 05666). USAID originally requested Dr. L. Lacey of the VBC, but prior commitments prevented his participation. On August 1, 1986, Dr. R. W. Lennox requested that I serve as a replacement consultant. My participation was approved by USAID/ Kathmandu (Ref: Kathmandu 06174).

My scope of work for Activity #AR-023 (August 18, 1986 agreement) was defined by the VBC as follows:

1. Attend VBC and USAID briefings in Washington
2. Accompany the NMEO team to the Far West
3. Provide entomological advice and assistance to the team regarding the design and implementation of short- and long-term studies on (1) incrimination of vectors, (b) possible role of outdoor malaria transmission, (c) vector ecology, and (d) alternative means of control
4. Review NMEO proposals for the Central and Far West Regions
5. Assess training requirements of mid-level NMEO staff
6. Submit a report acceptable to VBC, USAID and NMEO

My itinerary and activities for this trip are summarized in Appendix A and the persons contacted are listed in Appendix B.

II. EPIDEMIOLOGICAL AND ENTOMOLOGICAL OBSERVATIONS ON MALARIA IN KANCHANPUR DISTRICT

Description of the study area. Kanchanpur District lies in the newly formed Far West Malaria Region of Nepal and is bordered to the west and south by India (Fig. 1). Deforestation has created three ecological zones. These are: (1) large segments of the Terai in the south which have been cleared completely for agriculture and were planted in rice or corn at the time of the present survey; (2) a transition zone which consists of rice paddies and corn fields interspersed with remaining forest and is located at the base of the Churia range of hills to the north of the east-west highway; and (3) the slopes of the Churia range, which remain heavily forested. Agriculture to the north of the range is restricted to cleared river valleys of the Mahakali River drainage. Villagers reside for varying periods within the forest for woodcutting and cattle grazing.

Rainfall at Mehandranagar is heavy (1985 total = 1751 mm), and largely confined to the monsoon period (June-September) (Fig. 2). Temperatures are warm throughout the year (range of monthly means = 14.7^{o} to 30.6^{o} C) and allow perennial malaria transmission.[5] Humidity averages 40% during the pre-monsoon season, but increases to 80% during the monsoon season.

Epidemiology. The number of malaria cases detected by NMEO surveillance activities has increased markedly since 1983, and during 1985, a 8,381 total cases were detected in a population of 213,330 (API = 39.3/1000). Most infections were diagnosed as *P. vivax* (63.9% of total) and were considered to be contracted indigenously (92.6). Transmission occurred mostly during the monsoon season when 66.9% of the cases were detected (Fig. 3). Despite improved spray coverage and the use of radical treatment during the spring of 1986, the number of cases has remained high through July 1986. However, during the first 12 days of August and September, only 155 cases were detected, indicating a sharp decline in new cases. Reduced transmission occurred concurrently with an unseasonal decrease in rainfall (rainfall data unavailable).

Kanchanpur District has been subdivided geographically into five malaria units (Fig. 1). The highest case incidence occurred in riparian settings in Unit III to the north of the Churia range (Table 2); however, this Unit was only accessible by foot during the present survey (distance = 30 to 40 km). Observations during the current visit thus were confined to villages in Units II and IV having a high number of recent cases.

Epidemiological investigation of 61 recent cases from three villages in Units II (Sukasol) and IV (Bakun and Gauji) revealed that 41 cases (67.2%) were attributable to either relapse or recrudescence, 16 cases (26.2%) were imported from India (mostly Delhi area), and four cases (6.6%) were due to indigenous transmission. Case classification was by interview of the patients and was not confirmed immunologically. The high proportion of P. vivax relapses (67.2%) was higher than reported by the district office during 1985 (5%) and most likely was due to presumptive treatment using only chloroquine during 1985. The relatively high proportion of imported cases detected during the present survey (26.2%) was significantly higher than reported by the district office (0.6%) and emphasized the importance of careful case classification. The present survey indicated that few new cases during August and September 1986 were attributable to active malaria transmission.

Each of four recent indigenous P. falciparum cases which did not respond to chloroquine therapy were found to be drug resistant using WHO in vitro testing procedures. Drug resistance had been documented previously for cases imported from India, but had not been demonstrated for indigenous malaria cases in the western regions. In vivo evaluation was currently underway to classify the degree of resistance.

Entomological observations. Eight villages in Units II and IV comprising the three ecological zones were chosen for entomological study (Table 2). Villages were selected for study because of accessibility, occurrence of recent P. falciparum cases (Haldu Khal, Sukasol, Bakun), unsprayed status (Ulta Kham, Bandarpur), and/or ecological suitability (Muse Pani, Amthala Goth).

Mosquitoes were collected resting in houses or cattle sheds during early morning hours (0630-0800) using mouth aspirators for timed periods. Mosquitoes resting outdoors in natural refugia (crevices, vegetation, erosion furrows, etc.) were collected by mouth aspirator during morning hours (0700-1200). An attempt was made to standardize outdoor sampling by using artificial resting stations (clay water pots) placed in riparian, erosion gully and peri-domestic habitats (n = 3 jars/habitat). Host-seeking females were collected on human and bovid baits from dusk to dawn. Both NMEO personnel and local villagers were used as human bait during continuous all night collections. Animal bait collections were not standardized regarding collection effort or the numbers of baits used.

Anopheline adults were identified according to species, counted, and classified according to metabolic status (unfed, fresh fed, half gravid and gravid). Samples of females were dissected to determine follicular development (Christophers' classification scheme) and oviparity (dilation method of Polovodova using Hitchcock's vibrating needle dissection technique). Most females were dissected for sporozoite infection with the excised salivary glands examined without stain at 400X. Representative samples of An. annularis, An. culicifacies, An. fluviatilis, An. maculatus and An. subpictus females with ovaries at follicular Stage III were preserved for cytogenetic evaluation and will be presented at a later date by NMEO. The stomach contents of freshly fed resting females were smeared onto filter paper and will be sent by NMEO staff to Imperial College Field Station at Silwood Park for precipitin testing to determine host selection patterns (results to be presented later).

A total of 2,830 female anophelines comprising nine species were collected from Kanchanpur District during August 21-September 8, 1986 (Table 3). Most specimens (78%) were collected during early morning indoor resting catches. An. subpictus was most abundant, followed by An. culicifacies and An. vagus. All three species are known to be resistant to DDT in Nepal[6] and were equally abundant in sprayed and unsprayed villages in the rice growing zone. An. annularis and An. fluviatilis which are partially endophilic were not collected resting indoors in sprayed or unsprayed villages. Failure to collect An. annularis resting indoors was unanticipated, since this species reputedly is resistant to DDT.[6]

An. maculatus was the most abundant species collected resting outdoors (80% of total 91 specimens). The collection of An. culicifacies females at all reproductive stages resting outdoors indicated that this species is partially exophilic during the monsoon season in the Far West and could become progressively more exophilic if stressed by a change in insecticides. No mosquitoes were collected in the artificial resting sites (water jars).

Only five anopheline females (two An. culicifacies, one An. maculatus, and two An. subpictus) were collected biting man in 46 man-nights of collection effort. The low man biting rate of all anopheline species was unanticipated in light of the recent

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[6]White, G. B. 1972. Malaria receptivity stratification and projections for malaria vector control in Nepal. WHO/SEA/MAL/144, 66 pp.

malaria transmission at some of the houses used for all-night human bait stations at Haldu Khal, Gauji and Bakun. The use of NMEO or local baits made no difference in collection results. Nor did positioning the collectors in the forest and away from cattle increase the man biting rate. Biting rates were equally low in both sprayed and unsprayed villages. The low man biting rate was consistent with the apparent low rate of indigenous transmission as indicated by the epidemiological evaluation of recent cases.

Although not carefully standardized, more anophelines were collected biting bovid baits (520) than biting man (five) even though collection effort on bovid baits was considerably less intense. The zoophilic host selection patterns of anophelines in Nepal has been indicated during previous precipitin surveys.[6] Animal bait collections proved valuable for detecting the presence of An. annularis, An. nigerrimus, and An. peditaeniatus, which were not recovered using other sampling methods.

A total of 58 An. annularis, 88 An. culicifacies, 149 An. maculatus, and 287 An. subpictus were age-graded as to parity status (Table 5). The duration of the gonotrophic cycle for An. maculatus was considered to be three days, since sacculate females were not collected while biting. Sacculate females of the remaining three species were collected while biting and the duration of the gonotrophic cycle was considered to be 2.5 days, in agreement with White.[6] All species had a daily survivorship rate greater than 0.8. An. maculatus had the highest survivorship rate (0.92) and had the greatest number of females that had completed two or more gonotrophic cycles. Of epidemiological interest were the high percentages of An. annularis (33%), An. culicifacies (57%) and An. subpictus (61%) that were collected attempting to refeed at bovid hosts and that had primary follicles already developed to or past Christophers' Stage IV (Table 5). These females were exhibiting gonotrophic discordance and were attempting to refeed during the current gonotrophic cycle. The percentage observed seemed extraordinarily high and would certainly enhance the vectorial capacity of these species by increasing the frequency of host contact.

A total of 2,060 of the 2,830 anophelines collected (73%) were dissected to determine sporozoite infection, with negative results (Table 4). Negative findings were not surprising, considering the low man biting rates, reduced evidence of current malaria transmission, and the reduced abundance of the confirmed vector species (An. annularis, An. fluviatilis) during the present survey. The difficulty of directly incriminating anopheline vectors by recovering sporozoite infections in nature

is exemplified by the previous dissection records in Nepal, summarized by White.[6] To incriminate An. annularis, for example, 131,826 females were dissected during 1970 - 1981.

An. culicifacies females from an unsprayed village were tested for susceptibility to DDT and females from a DDT-sprayed village were tested for susceptibility to malathion (Table 6). Mortality after one hour exposure to critical concentrations of DDT and malathion and a 24-hour holding period were 26% and 99%, respectively. These data indicate that DDT-susceptible individuals and malathion-resistant individuals persisted in the population tests. The survival of one female exposed to 5% malathion needs confirmation. The few An. annularis that were inadvertently included with the An. culicifacies in the test cages were fully resistant to DDT and were partially resistant to malathion. A foothill population of An. maculatus which had never been pressured with insecticides was completely susceptible to both DDT and malathion.

Conclusions: An. fluviatilis, the reputed primary vector of malaria in the Terai, was collected infrequently in all three ecological zones during the present survey. As pointed out by Pradhan,[5] the seasonal abundance pattern of this species in the Far West during 1964 was asynchronous with the increase in malaria cases in August and September 1985. Further longitudinal research is needed to: (1) describe the seasonality of this species in the three ecological zones, (2) verify endophilic resting and endophagic biting behavior to justify the use of residual house spraying as a primary control method, and (3) verify the susceptibility of this species to DDT. It would seem unlikely that the persistent malaria transmission observed during the present survey could be attributed to An. fluviatilis at the present abundance levels and human biting rates (<0.02 bites/man/night).

An. annularis, a second but less efficient incriminated malaria vector in Terai, also was collected infrequently during the present study biting at bovid bait. Specimens were not collected resting in houses or cattle sheds, and thus it would be anticipated that the residual house spray program would have little impact on malaria transmitted by this species.

An. maculatus, recently incriminated as a vector of malaria in forested areas of the Central Region, was the most abundant anopheline biting bovid bait in the forested zone of the Churia range. Specimens were collected resting in the forest, but not biting bovids or man, in the transitional zone. Residual DDT house spraying would be anticipated to have little impact on

malaria transmitted by this species.

Extensive indoor resting collections in houses and cattle sheds in both sprayed and unsprayed villages indicated that An. culicifacies was the most abundant potential vector species. Although 66,420 females have been dissected for salivary gland sporozoite infection in Nepal with negative findings, these results are not entirely surprising considering the low vectorial capacity of this epidemic malaria vector in the outer Terai.[6] ELISA or similar sporozoite detection systems which permit the rapid evaluation of large numbers of specimens may prove useful in future incrimination attempts. Low-level exophily, resistance to DDT, and low-level resistance to malathion will require continuing entomological evaluation of the impact of ongoing residual spray operations.

Recommendations: The present short-term survey elucidated several areas which will require long-term longitudinal study before definitive recommendations on vector control can be made. Research should emphasize an evaluation of the spray program's impact on anopheline ecology and vectorial abundance. These research problems have been addressed by proposals submitted by NMEO staff (see section entitled, "Review of Research Proposals"), and specific methodology will not be presented in this section.

Table 2. Malaria Units of Kanchanpur District

Unit No.	Habitat type	Pop. Size	Malaria cases (1986) P.v.	P.f.	mix	TOT	Recent cases* P.v.	P.f.	TOT
I	terai, forests	64,628	946	44	1	991	18	0	18
II	terai, forests	54,707	1,727	83	1	1,811	62	2	64
III	forested hills	23,419	2,039	121	1	2,716	41	4	45
IV	terai, forests	56,310	1,794	115	2	1,911	28	0	28
V	river valleys	14,266	149	13	1	163	no slides		

*Data from slides submitted during the two weeks just prior to the present survey.

Table 3. Description of Villages Used for Entomological Observations

Village	Unit No.	Spray Dates 1985	Spray Dates 1986	Pop. Size	Malaria Cases 1985 P.v.-P.f.	Malaria Cases 1986* P.v.-P.f.
Cleared rice zone						
Haldu Khal	II	6/1-10	5/4-15	3,535	109-58	97-6
Ulta Kham	IV	9/10-19	none	2,705	85-23	75-4
Bandarpur	IV	9/10-20	none	1,680	36-17	34-5
Sukasol	II	6/16-27	5/9-17	1,910	48-21	60-5
Transition forest/agriculture zone						
Bakun	IV	9/1-3	5/9-11	940	51-86	175-13
Muse Pani	IV	9/11-12	3/22-24	452	47-38	54-4
Gauji	IV	9/6-11	3/19-22	990	69-63	119-8
Forested foothill zone						
Amthala Goth	IV	none	none	?	?	?

*Through mid-August 1986.

Table 4. Anopheline Relative Abundance and Sporozoite Dissections at Eight Villages in Kanchanpur District, Nepal
August 21 - September 8, 1986

Village	Method (effort)*	Species	No.	No./MH	Dissections**
		AGRICULTURAL ZONE			
Haldu Khal	indoor (6.5)	An. culicifacies	13	2.0	7
		An. subpictus	85	13.1	43
		An. vagus	6	0.9	1
	biting man (6)	An. culicifacies	1	0.2	0
	biting bovid	An. annularis	17		10
		An. peditaeniatus	1		1
		An. nigerrimus	8		8
		An. subpictus	49		34
		An. vagus	8		5
Ulta Kham	indoor (6.5)	An. subpictus	120	18.5	34
		An. vagus	4	0.6	0
	biting man (2)	An. subpictus	1	0.5	0
	biting bovid	An. annularis	26		26
		An. culicifacies	1		1
		An. subpictus	18		18
		An. nigerrimus	1		0
Bandarpur	indoor (4.5)	An. culicifacies	8	1.8	6
		An. subpictus	248	55.1	50
		An. vagus	13	2.9	7
	outdoor (1)	no mosquitoes			
Sukasol	indoor (4.5)	An. culicifacies	10	2.2	10
		An. subpictus	68	15.1	31

Table 4. (cont.)

Village	Method (effort)*	Species	No.	No./MH	Dissections**
Bakun	biting man (9)	no mosquitoes			
	biting bovid	An. annularis	16		16
		An. culicifacies	17		13
		An. fluviatilis	7		7
		An. nigerrimus	1		0
		An. peditaeniatus	5		0
		An. subpictus	12		9
		An. vagus	4		0
TRANSITIONAL AGRICULTURAL/FOREST ZONE					
Muse Pani	indoor (42.2)	An. culicifacies	313	7.6	319
		An. subpictus	306	7.3	100
		An. vagus	61	1.4	61
	outdoor (64.9)	An. culicifacies	8	0.1	8
		An. fluviatilis	1	<0.1	1
		An. maculatus	60	0.9	60
		An. subpictus	5	0.1	5
	biting man (14)	An. culicifacies	1	0.1	0
	biting bovid	An. annularis	3		1
		An. culicifacies	22		9
		An. fluviatilis	1		0
		An. maculatus	1		0
		An. nigerrimus	1		0
		An. subpictus	26		15
		An. vagus	7		6
Gauji	indoor (39.5)	An. culicifacies	424	10.7	422
		An. subpictus	437	11.1	374
		An. vagus	93	2.4	73

Table 4. (cont.)

Village	Method (effort)*	Species	No.	No./MH	Dissections**
Gauji (cont.)	outdoor (36.8)	*An. culicifacies*	1	<0.1	1
		An. fluviatilis	1	<0.1	1
		An. maculatus	13	<0.4	13
		An. splendidus	1	<0.1	1
		An. subpictus	1	<0.1	1
	biting man (10)	*An. maculatus*	1	0.1	1
		An. subpictus	1	0.1	1
	biting bovid	*An. annularis*	11		10
		An. culicifacies	9		9
		An. maculatus	1		1
		An. subpictus	6		6
		FORESTED ZONE			
Amthala Goth	biting man (5)	no mosquitoes			
	biting bovid	*An. annularis*	6		4
		An. culicifacies	10		7
		An. fluviatilis	6		0
		An. maculatus	202		108
		An. subpictus	12		9
		An. vagus	3		0

*Effort = man hours for resting catches or man-nights for human bait catches.
**Number of females dissected from each village for salivary gland infection with negative findings.

Table 5. Reproductive Status and Survivorship of Anophelines Collected Abundantly at Kanchanpur District, Nepal August 21 - September 8, 1986

Species	Collection Method	Follicular stage[1] I	II	III	IV	V	Parous[2] %(n)	S[3]	Dilatations[4] 0	1	2	3	4	5
An. annularis	biting	2	18	8	3	11	64(58)	.83	7	7	1			
An. culicifacies	indoor	1	10	13	7	2	76(42)	.89	8	9	1			
	biting	1	10	3	17	2	70(46)	.87	10	16	0	1		
	total						73(88)	.88	18	25	1	0		
An. maculatus	biting	0	121	19	1	0	77(141)	.92	33	84	14	8	1	1
An. subpictus	indoor	3	29	38	67	38	63(198)	.83	67	86	15	0	1	
	biting	1	21	15	46	11	74(89)	.89	23	55	11			
	total						66(287)	.85	90	141	26	0	1	

[1]Number of females classified into each follicular development class based on the scheme of Christophers.
[2]Percentage of total females scored that were parous.
[3]S = constant daily survivorship estimated by the formulae of Davidson based on the proportion of parous mosquitoes where the duration of the gonotrophic cycle was estimated to be 2.5 days for all species except *maculatus* which was considered to be 3.0 days.
[4]Numbers of females scored as to numbers of dilatations (i.e., previous ovipositions).

Table 6. Susceptibility of Three Species of *Anopheles* to 4% DDT and 5% Malathion, Kanchanpur District, Nepal
September 6-8, 1986

Village	1986 Spray	Species	% Mortality (n) DDT Control	DDT 4%	Malathion Control	Malathion 5%
Ulta Kham	-	*An. culicifacies*	13(15)	26(65)		
		An. annularis	--	0(8)		
Gauji	+	*An. culicifacies*			3(26)	99(68)
		An. annularis			--	80(5)
Amthala Goth	-	*An. maculatus*	0(15)	100(60)	0(15)	100(30)

[1]Freshly fed females exposed to WHO test papers at the critical dosage for one hour followed by a 24-hour holding period.

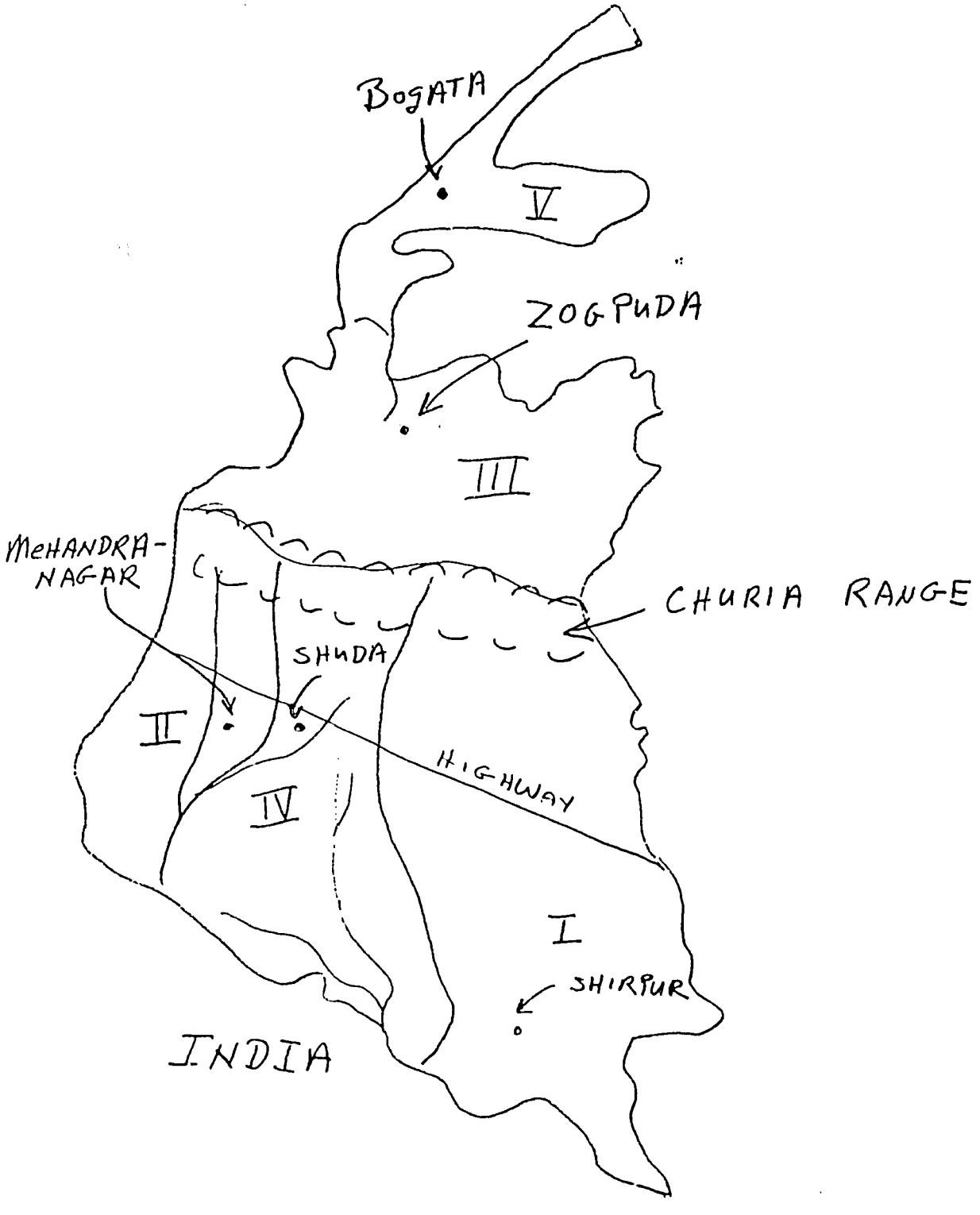


Fig. 1. MAP OF KANCHANPUR DISTRICT

FIG. 2. WEATHER AT MEHANDRANAGAR, KANCHANPUR DISTRICT, 1985

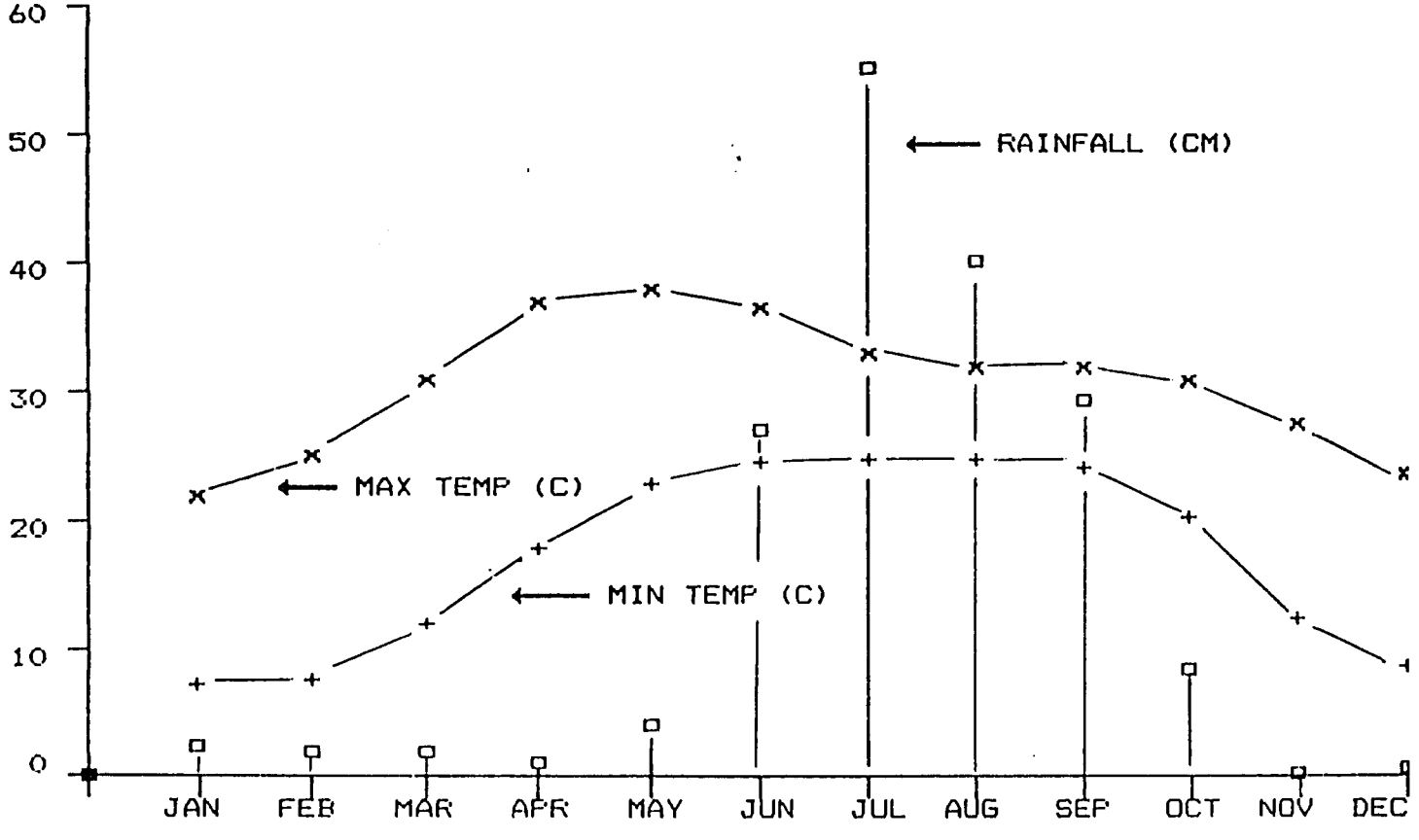
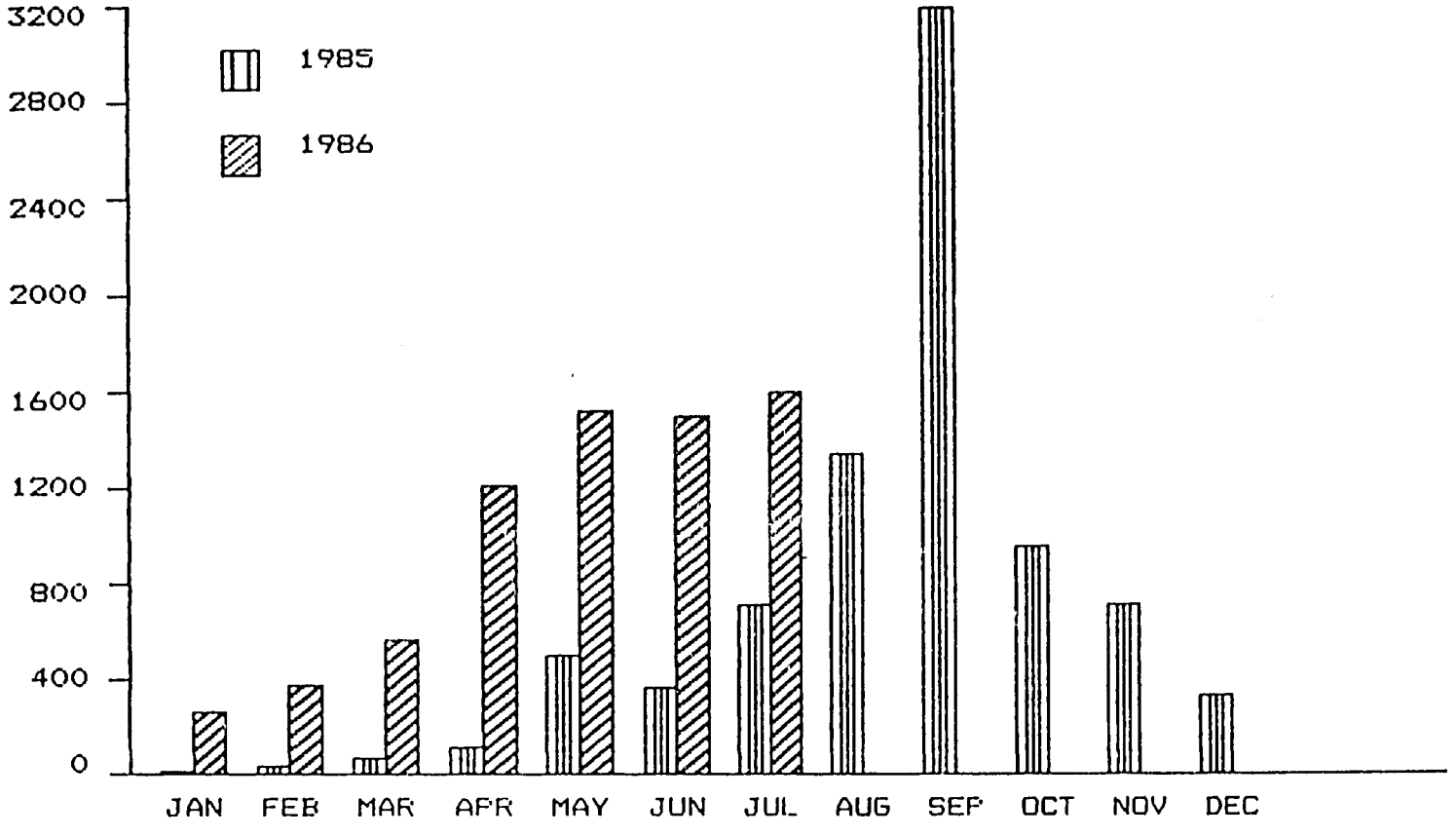


FIG. 3. MALARIA CASES IN KANCHANPUR DISTRICT



III. REVIEW OF RESEARCH PROPOSALS

Proposal 1. J. D. Shrestha. "Role of *An. fluviatilis* and *An. maculatus* in outdoor malaria transmission and its habit and behaviour in sprayed and unsprayed premises."

General Comments. The objective of the proposed research is to evaluate the role of the reputed anopheline vectors in the continued transmission of malaria despite residual house spraying with DDT (now Ficam in the Central Region). Specific research will focus on: (1) occurrence of outdoor transmission, (2) changes in mosquito biting and resting behavior associated with residual spraying, (3) evaluations of anopheline susceptibility to insecticides, and (4) considerations of alternative methods of control. The persistence of transmission despite ongoing control efforts most certainly warrants the initiation of entomological studies to evaluate the effectiveness of the current abatement strategy. The authors propose to leave portions of four study villages in two ecological zones unsprayed. I strongly suggest that the authors consider leaving one entire village unsprayed and one entire village sprayed in each ecological zone. The proposed data collection scheme would then evaluate the effectiveness of the spray program on the anopheline population ecology and vectorial capacity. I recommend that the proposal be funded after the authors consider the following specific comments.

Specific Comments:

P iii,5.1. No mention is made regarding how the study villages will be selected. I would suggest that in-depth epidemiological studies be conducted at the onset to verify the occurrence of active transmission despite ongoing control activities. Special epidemiological activities should be continued throughout the study to relate entomological activities to case occurrence.

Study sites within each village should be fixed and selected on the basis of mosquito relative abundance. A random selection of collection sites on each occasion would require considerably more sampling effort to account for sampling station variability. I would suggest that collecting trips be planned for two days duration and that two villages be sampled per week (blocked by ecological situation), i.e., two samples per month.

5.1.a. Authors should specify the time to be spent in each resting site and the number of premises to be searched. A total of 20 minutes (2 men for 10 minutes)/site x 4 sites x 2 biotopes = 160 minutes/sample should provide a sufficient index of indoor

resting abundance. Collections should be kept separate by site to provide an estimate of variability within a village as well as to allow comparison of the two biotopes. Collections should commence after sunrise to ensure that all females have ingressed and egressed.

5.1.b. This method is redundant to 5.1.a. and should be deleted.

5.1.c. Collections from animal baits should be standardized with one collector collecting from one bovid bait. This will permit the calculation of a man:bovid biting rate ratio, an additional index of host selection patterns.

5.1.d. Searches of outdoor shelters can substantiate exophilic resting habits; however, timed searches with mouth aspirators are inefficient, labor intensive and difficult to standardize. If possible, fixed sites near each indoor resting site should be established using one or more of the following sampling methods:

(1) four or more pit shelters should be dug on the outskirts of each study village and sampled concurrently with indoor resting catches;

(2) artificial resting units similar to the red boxes described by Goodwin (1944) could be placed on the outskirts of each village and sampled concurrently with indoor resting catches; or

(3) transects of representative vegetation could be established and sampled by mechanical aspirator.

5.2. Larval collections will most likely be qualitative but should be useful in describing the breeding habits of each species.

5.3.2. An attempt should be made to save some females for cytogenetic evaluation to verify the subspecific status of the populations within each study area.

External classification based on abdominal appearance should be verified by dissection and ovarian classification using the scheme of Christophers (1911). Disparity between abdominal appearance and ovarian development may help document the occurrence of gonotrophic dissociation or discordance during winter.

5.3.3. I would recommend either the age grading of empty females collected biting or a representative subsample of **all** females collected resting. Since the tracheation method can only be used on unfed females and a portion of the population of many Nepal anophelines refeed the same night as oviposition, the dilatation method of Polovodova will be required to age grade resting females. Dissectors should be required to pass a blind test on females of known reproductive history that have completed zero, one and two gonotrophic cycles. If the dilatation method is used, sample sizes should approach 100 females/species/month.

5.4. If data on the man biting rate, anthropophagic index, parity, duration of the gonotrophic cycle and temperature can be collected, I would recommend that vectorial capacity be calculated to quantify vector efficiency. The comparison of vectorial capacity and case occurrence can provide mosquito abundance thresholds at which to target control efforts.

The present protocol does not include methods for study of insecticide efficacy. I suggest that bioassays be conducted on sprayed surfaces during each visit to verify insecticide efficacy and persistence.

Protocol 2. Vaidya, R. "Bionomics and role of transmission of Anophelines in District Kanchanpur."

General Comments. Kanchanpur District has been experiencing a major outbreak of malaria. The proposal attempts to clarify the role of the local anophelines in transmission despite ongoing control by residual house spraying. However, the proposal lacks an epidemiological section which is needed to define carefully the malaria situation in the study villages during the entomological investigation. It would seem most valuable to NMEO to design research to evaluate the effectiveness of the ongoing DDT spray program for interrupting malaria transmission (especially because *An. culicifacies* and *An. annularis* are resistant to DDT in the Far West).

During a planning meeting with NMEO and USAID staff at Mehandranagar, it was agreed that Protocol 2 be revised to include the following major changes:

1. Objective: To describe entomological conditions which permit the continued transmission of malaria in Kanchanpur District despite residual DDT application. Studies will attempt to incriminate vector species by direct (salivary gland sporozoite infections) and indirect (comparative vectorial capacity estimates) methods.

2. Study areas: One unsprayed village will be selected for study in each of three ecological zones: (1) cleared rice growing area, (2) transitional forest/agriculture ecotone, and (3) uncut forest. A fourth sprayed village in either zone 1 or 2 will be studied to evaluate the impact of spray.

3. Epidemiological investigation should classify cases as 1) relapse, 2) imported or 3) indigenous. Authors should consider the use of immunological methods to classify cases objectively and thereby verify conclusions drawn from case interviews.

4. Mosquitoes will be sampled weekly at each village by a team of five collectors using the following methods:

   (a) Indoor resting: Two collectors will sample four houses, two mixed dwellings and two cattle sheds for 10 minutes starting 30 minutes after sunrise.

   (b) Outdoor resting: One collector will sample all anophelines resting in four or more pit shelters starting one hour after sunrise. Two collectors will search natural refugia to verify results from pit shelter collections.

   (c) Mosquitoes biting four men and one bovid will be collected from sunset to sunrise. Collections will be kept separate per hour to describe the biting rhythm of the abundant species in each ecological zone.

5. Processing: Mosquitoes will be transported to the Kanchanpur District Headquarters where they will be sorted as to species and sex and counted. Up to 25 females per species per collection method per village per week will be dissected to determine follicular development, oviparity, and sporozoite infection. The remaining females of the potential vector species will be dissected to ascertain sporozoite infection.

   The frequency of human blood meals among all blood meals will be determined by precipitin test for freshly fed females collected resting indoors and outdoors.

6. Indoor-resting females in the sprayed village will be tested for insecticide susceptibility to the residual spray compound. Spray efficacy will be evaluated by bioassay.

General Notes and Comments on Protocols: Every effort should be made to standardize carefully the methodology to be used in each protocol, thus allowing the direct comparison of results obtained in the Central and Far West Regions. Standardization is especially important for Protocol 2, where several NMEO staff entomologists will participate on a revolving basis. Mosquitoes should be collected at fixed and numbered sites and the data recorded on standardized forms to preclude inconsistencies related to staff rotation. NMEO needs to develop standardized pint-sized collection cartons and carrying containers for transporting the specimens to the laboratory for processing. Currently used test tubes are inadequate for the collection of large numbers of specimens, and the Barraud cages are space inefficient. The use of triethylamine as a mosquito anaesthetic may help processing, since this compound provides long-term knock down with minimal mortality if used in light doses.

IV. TRAINING ASSESSMENT

The NMEO National Malaria Training and Research Centre at Hetauda is currently staffed by instructors specializing in health education, parasitology, entomology and malariology. The Centre has planned to offer seven courses during fiscal year 1986/1987 to ca. 200 students (Table 7). The Centre supports the training and research needs for the NMEO 3,134 person staff and also provides malaria training for the Integrated Community Health Services Developmental Program (courses 5 and 6, Table 6). Basic courses (Table 6) last from six to eight weeks and may be supplemented by two-week refresher courses. Class size currently is limited by the small size of the lecture hall, limited number of microscope stations and number of field vehicles (one Land Rover for both research and training). A library essentially is nonexistent. Dormitory facilities are grossly inadequate.

Table 7. Course Titles, Student Educational Level and Numbers of Students Planned by the Hetauda NMEO Training and Research Centre for 1986

Course Title	Education Level (years)	No. Students
1. Malaria Inspector	10	30
2. Malaria Assistant	12	30
3. Lab Technician - microscopist	10	16
4. Vector control - malaria officer	14	16
5. Auxiliary Health Worker	10	30
6. Senior Auxiliary Health Worker	12	30
7. Statistics - malaria inspector	10	30

An insectary has been established using the artificial copulation technique. Colonies of An. annularis, An. fluviatilis and An. maculatus currently are being maintained by a staff of two technicians. Larvae are fed liver powder and adults are fed on human blood or chickens.

The 1985-1986 Centre budget for training is Rs 600,000. No funds are allocated specifically for research, which must be funded externally from the NMEO Headquarters for specific projects. The Centre staff was not actively involved in any research projects at the time of my visit. The Centre mosquito colonies have been used by the NMEO Central Region staff to study

the vector competence of Nepalese anophelines for imported chloroquine-resistant P. falciparum malaria. Improvement of research space and facilities would undoubtedly promote research to support NMEO control activities.

Although the Centre does provide training courses, the quality of the training and the response of the students would undoubtedly be enhanced by upgrading the Centre. Green et al. (1981)[7] have prepared detailed plans for upgrading the Centre at a 1980 cost of Rs 13,655,200.00 (US$ 650,000.00). The new facility would include improved teaching and research space as well as student dining and housing. At the current annual Nepal inflation rate of 14%, this facility currently would cost Rs 23,213,000.00 ($1,105,000.00) to build. As the quality of any disease control program is dependent upon the quality of the staff implementing the program, improvement of the Centre would seem the best way to maintain a cadre of well-trained and motivated staff. Improved facilities also would attract high quality teaching staff and stimulate research by NMEO staff as well as provide research space for visiting collaborating scientists.

The NMEO is proposing to expand its activities to become a vector-borne disease control organization responsible for diseases such as Japanese encephalitis (JEV), dengue, filariasis, leishmaniasis and murine typhus. The central location of the Centre at Hetauda within the Terai would seem well suited to the establishment of a national diagnostic facility capable of virus isolation and antibody determination. The plans proposed by Green et al.[7] could be modified to include arboviral laboratories and the Centre staff expanded to include individuals trained in virology. The current facility could not be upgraded to provide this capability. The Vector Biology & Control Project (VBC), Washington, should provide consultant support to modify existing plans for the Centre and ascertain manpower and training needs in vector-borne diseases other than malaria. Since NMEO personnel are already participating in JEV control, the VBC should organize short (four to six week) orientation courses for mid- and upper-level NMEO staff in four general areas:

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[7]Green, B. H., V. M. Rao, G. Sambasivan and J. D. Shastri. 1981. SEA/MAL/124, 90 pp.

1. Arbovirology: virus isolation and identification methods, virus-host interaction, virus life cycles, culicine mosquito identification, surveillance methods and sampling, control strategies applicable to Nepal.

2. Parasitology: introduction to filarial worms, identification of larval stages and microfilaria, surveillance methods, control methods, culture and identification of leishmania, phlebotomine identification, control methods.

3. Zoonoses: identification and ecology of ectoparasites, identification and ecology of small mammals, rodent control.

4. Vector control methods: emphasize integrated pest management approach applicable to Nepal, importance of mosquito surveillance.

Expansion of NMEO responsibilities will require serious support by the Government of Nepal to provide additional staff and laboratory facilities. Current NMEO staff and laboratory facilities cannot provide adequate surveillance and diagnostic support for JEV and leishmaniasis.

V. OBSERVATIONS ON THE JEV OUTBREAK IN NEPALGANJ

JEV recently has been recognized as a public health problem in the Terai region of Nepal.[8,9] From 1978 to 1980 there were 2,128 human cases of JEV with a 37% mortality rate. Animal sera samples (ducks, piglets, etc.) were 29% JEV positive (n = 187).

At the request of the USAID Malaria Advisor and selected NMEO staff, I was requested to comment on the ongoing JEV control activities. During my visit the Midwest Regional Headquarters in Nepalganj, I had the opportunity to observe JEV case management at Bheri Zonal Hospital and vector control operations by NMEO staff. During 1986, 14 cases of JEV have been diagnosed clinically, of which eight have died. Three cases were present in the hospital at the time of my visit and exhibited symptoms consistent with arboviral encephalitis. Unfortunately, acute and convalescent serum samples were not being collected, precluding laboratory confirmation. Spinal fluids were being tested for meningococcal infection. A superficial review of the case records failed to reveal a clumping of cases with regard to age (four to 70 years), sex (eight males/six females), or residence.

Ecologically the Nepalganj area seemed especially receptive to JEV maintenance. Surrounding rice fields and poor drainage within the city limits provided abundant breeding sources which superficially seemed suited to Culex vishnui-complex mosquito production. Wading (cattle egrets, herons) and perching (sparrows, etc.) birds were common in the rice field and urban environment. Pigs, an important amplifying host for the virus, were abundant and were being housed in residential areas. Thus, reservoir, amplifier and vector were present in close proximity to man.

NMEO personnel have been provided with six back-pack ULV sprayers and were applying ULV malathion in response to case occurrence. Spraying supposedly is done outdoors in early morning or evening and covers an area of about 50 houses surrounding the residence of the recent case. However, we observed ULV malathion application underway during mid-morning.

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[8]Katri, I. B., D. D. Joshi, T. M. S. Pradhan and S. P. Pradhan. 1983. Status of viral encephalitis (Japanese encephalitis) in Nepal. JNMA Souvenir, All Nepal Med. Conf. 11:97-111.

[9]Pradhan, S. P. 1984. A report of epidemiological investigation of Japanese encephalitis disease in three districts of Central Region of Nepal, 8 pp.

Encephalitis control using this approach most likely was doing more to appease than protect the population at risk, since the area near the case residences was sprayed two to three weeks after the transmission to man occurred and most likely after the infected female mosquitoes had already died or infected more of the population. Most likely, focal ULV applications in response to clinically diagnosed cases would have little impact on JEV transmission and should be discontinued.

Recommendations: JEV control may be approached prophylactically by immunization of the human population at risk or through vector control. Both approaches are costly with regard to manpower requirements and the outlay of foreign currency to purchase either the vaccine or the insecticides. Prior to initiating a large-scale control program, a careful definition of the scope of the problem would seem warranted using the following methods:

1. Acute and convalescent sera should be collected from clinically diagnosed JEV patients and sent to the Central Health Laboratory in Kathmandu for laboratory confirmation. The proportion of confirmed cases among clinically diagnosed cases will provide some indication of the reliability of clinical diagnosis and may reveal the presence of a presently unrecognized etiology. The distribution of the confirmed cases over time and space may define geographical areas at which to target surveillance and control activities.

2. Sero-epidemiological studies should be conducted in areas with a high number of confirmed JEV cases. Data will provide information on the ratio of apparent to inapparent infection and perhaps spatially delineate a segment of the population at which to target control activities. Veterinary information on equine disease and spontaneous swine abortion should be collected concurrently. Sera should be collected from ducks, pigs (less than one year old), chickens, and equines to determine which domestic animal population is infected most frequently and could be used in a sentinel surveillance program. Testing sera for a specific virus in a known host is far easier than isolating and identifying viruses from mosquitoes.

3. If continuing JEV transmission can be confirmed, an entomological survey should be conducted to determine, (1) what culicines are present and frequently feed on man and domestic animals, (2) the location and extent of the breeding habitats and (3) what control methods may most effectively interrupt transmission to man. Permanent

control measurers such as source reduction using improved drainage or water management are initially more expensive to apply but will yield longer lasting control and go far toward improving the general community environment. If mosquito population abundance cannot be controlled by larval measures, it may be necessary to establish a mosquito and virus surveillance system to forecast the onset of epizootic virus transmission. Emergency adult control measures can then be restricted in time and space to those populations at greatest risk.

4. The NMEO and USAID/Kathmandu should ask the VBC Project for external input on the careful design and implementation of a nationwide plan for JEV surveillance and control.

Appendix A. Itinerary for WKR Trip to Nepal August and September 1986

August 19	- Briefing by USAID/Washington.
August 21	- Briefing by USAID/Kathmandu.
August 22	- Briefing by NMEO Headquarters staff.
August 24	- Discussed the malaria situation in the Central Region and toured the Training and Research Centre in Hetauda.
August 25	- Discussed the malaria situation in the Western Region and discussed ongoing ICI insecticide trials in Brairahawa.
August 26	- Discussed the malaria situation in Danke District and the Mid West Region. Reviewed Japanese encephalitis virus (JEV) prevalence and control in Nepalganj. Examined JEV cases at Bheri Zonal Hospital.
August 28	- Discussed malaria situation in Kanchanpur District in Mehandranagar. Observed in vitro chloroquine resistance testing.
August 29	- Indoor resting collection at Gauji village. Dissected specimens to determine oviparity and sporozoite infections.
August 30	- Indoor resting collection at Haldu Khal village in the vicinity of recent malaria cases. Specimen processing.
August 31	- Indoor resting collection at the unsprayed village of Bandarpur. Specimen processing.
September 1	- Searched for outdoor resting anophelines and set out nine artificial resting units (ARU) (water jars) at Muse Pani village.
September 1-2	- All night bovid and human bait collections at Haldu Khal village.
September 3	- Checked ARU's and did evening (1900-2200 hours) bovid bait collections at Muse Pani.
September 4-5	- All night human and bovid bait collections at Amthala Goth.
September 6	- Helped set up and read <u>An</u>. <u>culicifacies</u> insecticide susceptibility tests for DDT and malathion.
September 7	- Helped set up <u>An</u>. <u>maculatus</u> insecticide susceptibility tests with DDT and malathion at Amthala Goth. Discussed ongoing trip results with USAID/Kathmandu.

Appendix A. (cont.)

September 8	- Discussed the malaria situation in Kailali District and the Community Health Leader Program in Dhangadhi.
September 9	- Debriefed USAID/Kathmandu and NMEO staff.
September 10	- Final briefing to USAID/Kathmandu in Delhi, India.
September 12	- Debriefed VBC and USAID/Washington staff in Washington.

Appendix B. Persons Contacted
(* = Kanchanpur field team member)

Washington
- Ralph Schmidt, VBC
- Larry Cowper, USAID

Kathmandu
- Barbara Spaid, Assistant Health Development Office, USAID
- Jay Anderson, Population Specialist, USAID
- Megh. B. Parajuli, Chief Officer, NMEO Headquarters
- *Shambu L. Shrestha, Deputy Chief Office, NMEO Headquarters
- *Jitendra P. B. Shrestha, Deputy Chief Office, NMEA Headquarters
- *Yeta Rajbhamdari, Entomologist, NMEO Headquarters

Hetauda
- *Janak D. Shrestha, Regional Malaria Officer, NMEO Central Office
- *Ganga L. Shrestha, Entomologist, NMEO Central Office
- Shambu L. Shrestha, Acting Chief Officer, NMEO Training and Research Center

Bhairahawa
- Rhiddi P. Vaidya, Senior Entomologist NMEO Headquarters and Acting Regional Malaria Officer, Western Region

Nepalganj
- A. R. Joshi, Regional Malaria Officer, Mid West Region
- Durga S. Mauendhar, Civil Surgeon, Bheri Zonal Hospital

Mehandranagar
- *Tekendra B. Swar, District Malaria Officer, Kanchanpur District
- *Dr. Mohd. Karim Nushin, WHO Malariologist

Dhangadhi
- Goviord P. Shrestha, District Malaria Officer, Kailali District
- Stebon Eddy, Peace Corps Volunteer, Community Health Leader Program

Appendix C. Attached Proposals Submitted by NMEO Staff (pencilled comments by WKR)