VECTOR BIOLOGY & CONTROL

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MALARIA IN NEPAL

DEVELOPMENT OF A DETAILED WORKING PLAN FOR ENTOMOLOGICAL RESEARCH IN SINDHULI (CENTRAL REGION) AND KANCHANPUR (FAR WESTERN REGION) DISTRICTS WITH COMMENTS ON JAPANESE ENCEPHALITIS

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by

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EXECUTIVE SUMMARY

At the request of USAID/Kathmandu and the Nepal Malaria Eradication Organization (NMEO), I visited Nepal during March and April 1987 as technical advisor to research projects planned for Sindhuli and Kanchanpur Districts. At the request of NMEO, I prepared a detailed working plan for the field research and provided comments on the Japanese encephalitis problem in terai.

Findings and recommendations. The NMEO research protocol was reviewed and suggested changes discussed with the NMEO co-investigators and the USAID Malaria Advisor. The principal investigator was not present during my visit.

A detailed working plan was written for field research in Sindhuli and Kanchanpur Districts. Included were (1) preliminary surveys of the study villages to assist in the development of a final sampling design; (2) detailed description of sampling methodology and specimen processing; (3) a schedule of sampling activities; (4) an outline of quality controls; and (5) a list of additional supply items required.

An orientation and training course for the intermediate level staff working on the field research projects was outlined and will be conducted at the Research and Training Centre in Hetauda. The course will include (1) preparation of ovaries for cytogenetic evaluation; (2) mosquito age grading; (3) sporozoite detection, (4) larval anopheline identification; and (5) record-keeping for the research project.

The previous Japanese Encephalitis Virus control plan of the Government of Nepal was reviewed. A three-phase program to provide mosquito control, surveillance and emergency response to epidemics was presented briefly.

1. INTRODUCTION

Continued malaria transmission despite ongoing control efforts by the Nepal Malaria Eradication Organization (NMEO) has stimulated the development of research protocols aimed at elucidating the entomological aspects of malaria transmission in Sindhuli and Kanchanpur Districts. Initially two proposals were developed by NMEO staff. NMEO was to fund staff and transportation, USAID/Kathmandu was to provide supplies, equipment, and technical assistance by the USAID Malaria Advisor, and the Vector Biology & Control (VBC) Project was to provide collaborative technical assistance through short-term consultancies. Proposal 1 by J. D. Shrestha was aimed at elucidating the causes for persistent malaria transmission in endemic areas of the Central Region, while Proposal 2 by R. Vaidya was directed at determining which Anopheles species were responsible for transmission during the recent epidemic of malaria in the Far Western Region. Both proposals described timely and much needed entomological research critical to ongoing control efforts by NMEO.

At the request of USAID/Kathmandu, the original NMEO proposals were reviewed with the NMEO research staff by Dr. W. K. Reisen, VBC consultant, during August 19 - September 1986.[1] These protocols were revised by S. L. Shrestha (Appendix A) and submitted to USAID/Kathmandu which, in turn, requested the VBC to provide a project consultant for the outlined research (Kathmandu 00475). In February 1987, the VBC Project asked me to participate collaboratively during three short-term (2-3 week) consultancies at the beginning, middle and end of the proposed 18-month research project. My work scope for the first trip (VBC Activity AR-026-2, March 18 - April 8, 1987) was to participate as a technical advisor to NMEO research projects in Sindhuli and Kanchanpur Districts by (1) reviewing the revised research protocol by S. L. Shrestha (Appendix A); and (2) accompanying the USAID/Kathmandu Malaria Advisor and NMEO staff to the proposed study areas to advise on sampling and study design. In addition, NMEO requested that the USAID Malaria Advisor and I prepare a final detailed working plan for the proposed research, based on the revised protocol (Appendix A) and on site discussions with the NMEO co-investigators. Comments on the Japanese encephalitis problem were requested by USAID and NMEO. My itinerary and list of persons contacted are summarized in Appendices B and C, respectively.

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[1]Reisen, W. K. September 1986. Malaria in Nepal: Observations during a short-term entomological consultancy. VBC Activity AR-023:33 pp.

2. REVIEW OF RESEARCH PROTOCOL

Title: Intensive entomological studies in hard-core areas of Nepal.

Principal investigator: S. L. Shrestha

General comments: The objectives of the proposed research are to determine why malaria transmission persists in the Central and Far Western Regions of Nepal and to study the biology of the anophelines in order to develop new, or modify ongoing, control strategies. The research is much needed and is justified by the paucity of current entomological data.

Combining the two original protocols into a single proposal has complicated the interpretation of study design. Research in Sindhuli District (Central Region) originally was designed to (1) determine the role of *An. maculatus* in outdoor transmission; and (2) describe changes, associated with insecticide pressure, in the resting/biting behavior and breeding site selection by *An. fluviatilis*. The present proposal will describe resting and biting behavior within the selected study villages, but fails to address the problem of outdoor transmission at cattle sheds (goths) in forested areas. In addition, the revised proposal calls for suspension of spraying activities in all study villages. This tactic will preclude the evaluation of the impact of residual insecticide spray on anopheline behavior.

The principal objective of the entomological research in Kanchanpur District was to determine which *Anopheles* species is responsible for the ongoing malaria epidemic. The proposal as designed will provide data to incriminate directly or indirectly the current vector species. The proposal states that residual spray will not be applied to the study villages and thus, the current research will not provide data to establish the cause(s) for persistent transmission in sprayed villages. The research also will not determine whether transmission is occurring at cattle sheds in the forest. In addition, no indication is given as to what control methods might be applied in a follow-up study. Every effort should be made to ensure that the current research will collect a data set amenable to future control method evaluation. Control strategies currently investigated elsewhere by NMEO include (1) strengthened surveillance alone (i.e., prompt diagnosis and treatment); (2) use of larvivorous fishes; and (3) environmental management.

Specific Comments on the NMEO Proposals

P4. The fortnightly collection schedule is justified scientifically by the research objectives, but will require the research staff to shift camp between villages on a weekly basis. The investigators might consider sampling during two consecutive weeks and then shifting to the next village. This would reduce the number of moves from 52 to 26 and still would provide the same number of replicate samples per month.

P4, 6.1.4. The protocol does not specify how the immature specimens will be identified. Will pupae be reared to adults in the field or will identification be based solely on larval specimens? Sampling could be expedited by the use of sample concentrators which would preclude the need for laborious transfer of individual specimens in the field using a pipette. In addition, it would be more useful to survey the entire village and its immediate environs for larval breeding each month than to rely on fixed permanent breeding sites. Sites chosen at the onset of the study during the dry season might not be the most important mosquito production sites in the village during the monsoon, especially if some species prefer to oviposit in newly created breeding sources.

P4, 6.1.5. If parity determination will be made using the method of Detinova (presence or absence of tracheal skeins), I would prefer that dissections be restricted to the biting population. Since many anophelines refeed during the same night that they oviposit, dissections of only the unfed resting females may lead to serious underestimation of the parity rate and thus, estimates of survivorship and vectorial capacity. The estimate of parity should be based on no less than 50 females/species/month.

P5, 6.1.8. I did not understand why susceptibility tests were conducted seasonally when none of the villages were being sprayed. I think that knowledge of the susceptibility status of the population to the previous control regimens would provide useful background information, but that continued testing without additional insecticide pressure was not warranted.

3. DETAILED WORKING PLAN FOR EXTENSIVE ENTOMOLOGICAL STUDIES

Title: Entomological studies to elucidate the causes for persistent malaria transmission in Sindhuli (Central) and Kanchanpur (Far Western) Districts.

Duration: 18 months, starting June 1, 1987.

Principal investigator: S. L. Shrestha

Co-investigators: J. P. B. Shrestha, J. D. Shrestha, S. R. Shrestha and Chiranjebi Shah

Collaborators: S. P. Pradhan, USAID Malaria Advisor and W. K. Reisen, Vector Biology & Control Project

Executing organization: Nepal Malaria Eradication Organization.

3.1 General objective

To elucidate the entomological causes for continued malaria transmission in the hard-core areas of the Central and Far Western Regions of Nepal and to recommend appropriate control methods.

3.2 Background and specific objectives

3.2.1 Central Region

Epidemiological investigation of selected malaria cases in the inner and outer terai of the Central Region indicated that transmission was occurring outdoors in the forest at wood cutting camps or cattle sheds (goths) and away from the village environment. Both *An. fluviatilis* and *An. maculatus* were found to be infected with sporozoites during subsequent entomological surveys conducted during June 1985 and December 1986, respectively. These data provided the first incrimination of *An. maculatus* as a vector of outdoor-transmitted malaria in the terai. However, since transmission appeared to be occurring concurrently in the village environment, it was not possible to determine the actual reservoir of infection or direction of the transmission, i.e., whether the villagers brought the malaria parasites from village foci to the forest where the forest *Anopheles* became infected, or the villagers became infected at the goths from forest *Anopheles* infected by gametocytemic transients and then brought the malaria back to the village where secondary transmission occurred. The sporadic occurrence of *P. falciparum* seemed to support the latter hypothesis. The situa-

tion was further complicated by the low house-resting densities of the primary malaria vector, An. fluviatilis, which reputedly was still susceptible to both DDT and Ficam.

Possible hypotheses to explain persistent malaria transmission included:

1. Transmission was occurring outdoors in both the forest and the village habitats;

2. An. fluviatilis endophilic and/or endophagic behavior was modified by residual spray application or irritability to DDT; and

3. Changes in the ecology of the terai modified the mosquito fauna; additional or other Anopheles were not serving as vectors.

To test the above hypotheses, an 18-month longitudinal study is proposed with the following objectives:

1. Determine the importance of outdoor transmission by forest anophelines, including An. fluviatilis and An. maculatus, in the maintenance of malaria at representative villages in Sindhuli District;

2. Evaluate the impact of DDT residual house spray on anopheline behavior and susceptibility status; and

3. Elucidate the role of possible secondary vector species in malaria transmission.

3.2.2 Far Western Region

Malaria is currently epidemic in Kanchanpur District with annual attack rates of 39.3 and 41.9 per 1000 occurring during 1985 and 1986, respectively. Case prevalence were highest during September 1985 and July 1986, periods when the abundance of the reputed vector, An. fluviatilis, was minimal. A short-term entomological survey during August and September 1986 (1) failed to incriminate directly any of the Anopheles species by sporozoite dissection; (2) found that the man-biting rate was very low (< 0.5 bites/man/night); (3) demonstrated that An. culicifacies, An. subpictus and An. vagus were the most common species collected resting in houses in sprayed and unsprayed villages; and (4) indicated that An. maculatus was the most abundant

species in the forested foothills.[1] Epidemic malaria transmission in the absence of abundant populations of the primary (An. fluviatilis) or secondary (An. annularis) vector species indicated that other species, such as An. culicifacies (the primary vector of malaria throughout the Indo-Pakistan subcontinent and Sri Lanka), may be playing an important role in the current epidemic. Because An. culicifacies is resistant to DDT, abatement strategies, therefore, would have to be modified to include alternative insecticides.

Continued high malaria attack rates despite concerted control efforts highlighted the urgent need for in-depth research to test the following hypotheses:

1. Deforestation and insecticide pressure have changed the anopheline fauna of Kanchanpur District, eliminating or reducing the abundance of the primary malaria vector, An. fluviatilis; and

2. Malaria is not maintained by a new complex of anopheline species.

To test the above hypotheses, an 18-month research project is proposed with the following specific objectives:

1. Comparatively describe the bionomics of the anopheline fauna inhabiting the three distinct ecological zones of Kanchanpur District where malaria is endemic: deforested agricultural zone, newly formed agriculture/forest ecotone, and forested foothill zone;

2. Determine the potential vectorial role of each Anopheles species in the maintenance of the ongoing malaria epidemic; and

3. Determine the susceptibility status of the common Anopheles to DDT, malathion and Ficam.

3.3 Description of study villages

3.3.1 Sindhuli District

Two representative inner terai villages (T. Ranibas and T. Kutepani) situated along the southern bank of the Kamala Nadi were selected for longitudinal study (Table 1). T. Ranibas had a

[1]Reisen, W. K. September 1986. Malaria in Nepal: Observations during a short-term entomological consultancy. VBC Activity AR-023:33 pp.

TABLE 1. POPULATION AND MALARIOMETRIC CHARACTERISTICS OF THE STUDY VILLAGES SELECTED FOR STUDY IN SINDHULI AND KANCHANPUR DISTRICTS

Study Village	Year	Population size	Number Cases P.v.	Number Cases P.f.	Spray
Sindhuli District					
T. Ranibas	1985	905	130	0	DDT - 2 rds
	1986	965	41	1	Ficam - 3 rds
T. Kutepani	1985	277	17	0	DDT - 2 rds
	1986	230	5	0	Ficam - 3 rds
Kanchanpur District					
Badaipur	1985	1385	42	10	DDT - 1 rd
	1986	1373	32	2	DDT - 2 rds
Musepani	1985	452	47	40	DDT - 1 rd
	1986	719	82	9	DDT - 2 rds

higher malaria case rate during 1986 (42.4/1000) than did Kutepani (21.7/1000) and will be left unsprayed to allow the study the dynamics of malaria transmission within the village environment. Sampling will be concentrated along transects paralleling the Kamala Nadi with transect 1 adjacent to the river bank and transect 2 at the southern margin of the village near the forest ecotone at the base of the Churia hills. Kutepani will be sprayed twice (May and September) with DDT (1 gm/m^2) to study the impact of spraying on anopheline behavior and infection rates. Sampling effort in Kutepani will be divided among the two major clumps of houses on the west and east ends of the village. Additional observations for study of the ecology of forest malaria transmission will be made in the forest at goths used by villagers residing in T. Ranibas or T. Kutepani.

3.3.2 Kanchanpur District

Entomological observations will be made at three representative localities in each of three ecological zones: Badaipur- a village in the deforested agricultural zone; Musepani - a village at the agriculture/forest ecotone; and Musepani goth - a forested cattle-grazing area in the Churia hills (Table 1). Villages will be left unsprayed during the first 12 months of study. Control may be attempted during the second summer. Badaipur village is bordered to the west by the Mahakali River and to the east by the Sukla-Phant National Park. Sampling will be concentrated along two parallel east - west transects perpendicular to the Mahakali River. Musepani is bordered on the west by the Musepani Nadi, on the east by a series of seepage rivulets and on the north by the forested Churia hills. Sampling effort will be concentrated along north - south transects near the Musepani Nadi and adjacent to seepage streams starting at the forest interface.

3.4 Epidemiological Observations

The NMEO district staff will conduct monthly active case detection (ACD) at each study village. In Kanchanpur District, ACD activities will augment the current malaria depot system. Positive slides collected from patients in the study villages by both ACD and passive case detection systems will be saved for future examination to determine accurately the number of gametocyte carriers. All cases will be investigated epidemiologically to determine probable source of infection, date of onset, and relapse/recrudescence status.

3.5 Sampling Methods

Mosquitoes in each village will be sampled by an entomological team of eight NMEO staff members using the methods described below:

3.5.1 Indoor Resting Collections

Female anophelines resting inside eight fixed houses and eight fixed cattle sheds will be sampled for 10 minutes each by two collectors using mouth aspirators and flashlights (total collection effort = 5.3 man hours). Two teams of two collectors will sample all structures during a 2 - 3 hour period starting at dawn. An additional two houses and two cattle sheds will be inspected randomly to ensure that the fixed catching stations are providing representative information on anopheline abundance. Special collections also will be made at the residences of recent fever cases. Collections will be kept separate according to structure to provide replication for planned analyses.

3.5.2 Outdoor Resting Collections

Two teams of two collectors will sample outdoor resting anophelines concurrent with efforts to collect indoor resting anophelines (i.e., for 2 - 3 hours starting at dawn on the same day that indoor resting samples will be taken). Samples will be taken from four permanent pit shelters (1.5 m x 1.5 m x 1.5 m) fitted with a canopy to minimize flooding and with four 30 cm^3 holes, one on each side of the shelter. In addition, natural refugia along stream banks and river beds will be searched for outdoor resting anophelines. Collections will be kept separate according to pit shelter and major outdoor habitat type, such as tree roots along river bank or erosion furrows.

3.5.3 Human Bait Catches

Three teams of two collectors will collect all anophelines attempting to feed on their exposed extremities from dusk through dawn (collection effort = six man-nights per sampling interval). One collector from each team will collect mosquitoes indoors, while the second collector will be seated on the veranda or in the courtyard. Collections will be made continuously without a meal break. Additional biting catches may be performed at residences where fever cases occurred recently. Specimens will be kept separate according to collector and location (i.e., house number and indoor or outdoor collection site).

Goths will be sampled by a team of two collectors and one porter in conjunction with collections at Kutepani and Muscpani. Only human baits will be used and specimens will be transported by motorcycle to the study villages for processing.

3.5.4 Bovid Bait Collections

A team of two collectors will aspirate all anophelines attempting to feed on a single, tethered bovid bait (preferably a female buffalo). One bait will be positioned inside a cattle shed, and the second will be positioned outdoors. Every attempt will be made to utilize the same baits during each sampling effort. Collections will be kept separate by hour from dusk to dawn and by bait location. Catches will be made continuously for the first three hours after dusk and then for 30 minutes per hour for the remainder of the night (number of mosquitoes per hour will be calculated by multiplying x 2). COLLECTIONS SHOULD BE MADE ONLY FROM THE BOVID HOSTS AND NOT FROM THE SURROUNDING CATTLE SHED OR OTHER ANIMALS.

3.5.5 Larval Collections

Three teams of two collectors will survey the entire village for groundwater breeding sites positive for Anopheles larvae. Each breeding site will be numbered, plotted on a sketch map of the village, and notes recorded as to the approximate size, depth and vegetation. A total of 25 dips will be made throughout the breeding site, pooled in a sample concentrator (Appendix D) and then transferred to a labeled collection jar. The total numbers of larvae and pupae in each sample will be counted. Pupae will be transferred to emergence chimneys and the resulting adults identified by species and counted. The larvae will be killed with hot water and preserved in vials with McGregor's solution for future identification.

3.6 Sampling Processing

Mosquitoes will be processed immediately after collection or kept alive until time permits completion of dissections. Abdominal and ovarian classification should be made as soon as possible after collections. Salivary gland dissections, however, may be delayed.

3.6.1 Resting Collections

All indoor- and outdoor-resting anophelines will be sorted as to species, sex and female metabolic state, and counted. Metabolic states include the following:

- unfed - no blood in the gut and follicles at Stages N-IIa[2]
- freshly blood fed - red undigested blood visible in the gut and follicles at Stages II or III
- half gravid - half of the abdomen filled with black digesting blood and half filled with developing follicles at Stages III or IV
- gravid - blood meal completely digested and the abdomen filled with Stage V follicles

Up to 50 specimens of each Anopheles species per month per village from both indoor and outdoor collections will be tested for the presence of human blood. Females that are freshly blood fed will be smeared into filter paper and then transported to Kathmandu for identification using the gel diffusion technique.

All females will be evaluated for the presence of sporozoites. In Sindhuli District, emphasis will be placed on the dissection of the reputed vectors of malaria (An. fluviatilis and An. maculatus), although other species commonly collected also will be dissected. In Kanchanpur District, the first 50 females/species/collection method (i.e., indoor or outdoor) will be dissected using conventional methodology. The remainder of the specimens will be evaluated in pools of $\leq$25 females using the sporozoite concentration method. Pools will be ground in a tissue grinder in saline solution, centrifuged and the buffy coat examined with Giemsa stain for the presence of sporozoites.

3.6.2 Biting Collections

All females collected biting will be identified by species and counted. All females collected at human bait will be dissected for both gut and sporozoite infection. Up to 20 females/species/hour collected biting bovid baits will be dissected to determine reproductive status. Ovaries will be excised into physiological saline and the follicular stages noted using the scheme of Christophers.[2] One ovary will be placed on a separate slide for later parity determination using the tracheation method of Detinova.[3] Ovaries will be placed on

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[2]S. R. Christophers. 1911. The development of the egg follicle in anophelines. Paludism 2:73.

[3]Detinova, T. S. 1962. Age-grouping methods in Diptera of medical importance. WHO Monogr. 47:1-210.

slides numbered to coincide with the dissection record sheet and retained for later verification. The second ovary will be disrupted and examined under 400x for the presence of dilatations and sacculate follicular pedicels as described by Detinova.[3] The guts and salivary glands of the same females also will be examined for Plasmodium infection. In Kanchanpur District, extra specimens will be tested for sporozoite infection using the concentration method.

3.7 Insecticide Susceptibility

All abundant anopheline species in all villages will be tested for resistance to DDT, malathion and Ficam using standard WHO procedures and test kits. One complete test will be completed at the onset to provide baseline information on the resistance status of each common species. In Sindhuli District, follow-up tests using DDT will be performed at both Kutepani and Ranibas villages prior to each spray round. If necessary, extra resting or biting collections will be made to provide sufficient specimens for the susceptibility tests. Irritability tests to DDT will be performed in Sindhuli District at the onset and repeated prior to each spray round if need is indicated by the data.

3.8 Meterological Data

Temperature and relative humidity will be recorded at hourly intervals by sling psychrometer during each all-night biting collection. Wind and rainfall will also be noted. Temperature range will be recorded by maximum/minimum thermometer on each day that the research team is in the village. Rainfall data will be obtained by gauges placed at the nearest malaria unit office. Data on crop planting, growth, acreage under cultivation and harvesting will be recorded for each village.

3.9 Work Schedule

3.9.1 Preliminary Observations

During April 1987, sketch maps will be prepared for each of the study villages in Sindhuli and Kanchanpur Districts. Maps will include house numbers, major rivers, canals and forested areas. A census of the entire village also will be conducted, with the numbers of humans and domestic animals recorded by family name and house number. The NMEO Unit in Charge will provide data on malaria case occurrence by house and/or family name during 1986, 1987 and throughout the course of the study.

During April 1987, the entomological teams at the study villages will sample indoor-resting mosquitoes from every house in the village (one man for 10-15 min/house or cattle shed). Collections will be kept separately according to by house number, and all Anopheles will be identified by species and sex, and counted. Early evening biting collections (dusk to 2200 hours) will be conducted at houses with high indoor resting abundance. The location of suitable bovid baits will be noted and early evening biting catches attempted.

Preliminary resting and biting data will be used to establish fixed sampling sites within each study village for indoor and outdoor resting catches and for all-night human and bovid biting collections.

NMEO will transfer personnel to the study villages to complete the research teams. Additional manpower requirements for Sindhuli District include one malaria inspector and one insect collector. For Kanchanpur District, additional manpower requirements include two malaria assistants, one malaria inspector and one insect collector. Entomologists, malaria assistants and malaria inspectors responsible for mosquito identification and specimen processing will report to the Research and Training Centre in Hetauda during the week of May 17, 1987 for final orientation and training by the USAID malaria advisor and senior NMEO staff. A tentative training schedule is outlined in part 4 of this report.

3.9.2 Daily Working Plan

Starting June 1, 1987, each study village will be sampled for two consecutive weeks according to the following schedule:

Sunday:	Transfer to study area and set up camp	
Monday:	A.M.	Indoor and outdoor resting collections
	P.M.	Specimen processing
Tuesday:	A.M.	Complete any leftover dissections, and rest
	P.M.	All-night human and bovid bait collections
Wednesday:	A.M.	Specimen identification and processing
Thursday:	Larval survey (six men); two men will travel to Goth for all-night biting collection	
Friday:	Identify specimens from the Goth collections, identify adults emerging from larval collections, complete collection records, do extra collections for susceptibility tests, etc.	

Saturday: Off

Monday to Friday: Repeat the above schedule at the same village

Saturday: Off

Sunday: Transfer to second study village

3.9.3 Additional Observations

Quarterly: The subspecific status of up to 25 specimens of the reputed or potential malaria vectors (especially An. annularis, An. culicifacies and An. fluviatilis) will be evaluated cytogenetically. Slides will be prepared by the field research staff and shipped to Kathmandu for identification.

Twice annually: The susceptibility to DDT of the common anophelines at both Kutepani and Ranibas will be determined prior to each spray round at Kutepani.

3.10 Quality Controls

NMEO senior staff, the USAID malaria advisor and the VBC consultant repeatedly will visit the field areas according to the attached schedule (Appendix E) to ensure data quality. In addition, the following preliminary experiments and controls will be required to ensure that the data collected is meaningful.

Identification. Field teams should be provided with insect specimen boxes to preserve a voucher series of each anopheline species they identify as well as unusual specimens they have trouble identifying. The cytogenetic determination of one or more sibling species in the study villages will require the frequent preservation of ovary slides for chromosomal examination.

Parity determination. Individuals routinely performing parity dissections should be required to pass a blind test on specimens of known reproductive history prepared by the insectary staff at Hetauda. A grade of >90% can be considered as passing. In addition, the preservation of the dried ovaries of unfed specimens on slides will allow the senior investigators to verify field parity determinations. Slides should be examined by senior staff during visits to the field areas. This control can be discontinued after the senior staff verifies 100% accuracy.

Sporozoite determination. The sporozoite concentration method should expedite specimen processing and assist in vector incrimination, especially for epidemic vector species such as An. annularis and An. culicifacies. The technique should be worked out completely in Hetauda using known infected specimens fed on gametocyte carriers. Infected females should be diluted with 10, 20, 30, 40 and 50 uninfected specimens to determine optimal pool size.

Larval identification. Permanent slides should be made of at least five specimens of all species identified as larvae by the field staff; these slides should be checked by the senior research staff. Questionable determinations should be verified by individual rearings for taxonomic study.

3.11 Data Reporting

All data will be entered daily on preprinted data forms. Data will be compiled weekly by the senior team member and submitted to the principal investigator for checking. The principal investigator will summarize the results quarterly and submit a written copy to each of the co-investigators, the USAID malaria advisor and to W. K. Reisen in California.

3.12 Additional Supplies

In addition to the original supply and equipment list, the following items are requested for facilitation of the field research project:

USAID/Kathmandu (local purchase):

1. Kanchanpur - Tents (4) - three two-man tents for sleeping and one four-man tent to serve as a field laboratory; camp cots (8)

2. Folding tables (2); folding chairs (8)

3. Mosquito nets (16)

4. Petromax lanterns (2)

5. Emergency lights, D-cell powered (6)

Vector Biology & Control Project:

1. Plastic petri dishes - 100 mm (144), 25 mm (144)

2. Plastic urine sampling bottles (144)

3. Dissection supplies - applicator sticks, minutes nadelines, watchmaker forceps (10 sets)

4. One pt Fonda paper cans (case of 200) and six rolls of duct tape to make mosquito cages

5. Insulated styrofoam shipping boxes with straps (6)

6. Triethylamine for mosquito anesthetization (6 pts)

7. CDC style battery-powered miniature light traps (4)

4. OUTLINE OF ORIENTATION AND TRAINING COURSE AT HETAUDA

4.1 Purpose

The objectives of the proposed week-long orientation and training course at Hetauda for intermediate level research staff are to (1) outline the rationale behind the proposed research, emphasizing the importance of the data to the NMEO, (2) describe the research techniques that will be used, (3) provide training for the persons performing the research activities, including "hands on" practice in the laboratory with known controls, and (4) ensure that the record-keeping and reporting procedures are understood.

4.2 Tentative Schedule

Training at Hetauda is tentatively scheduled from May 18-22, 1987 with the following daily activities:

May 18 - A.M. Objectives of the proposed research and relation to NMEO program. Introduction to the research techniques used from direct and indirect vector incrimination. Students should be given a copy of the research proposal to read during the ensuing week so that they can ask questions about topics including methods, record keeping, and scheduling.

P.M. Mosquito identification and the subspecies concept. Learn to make ovarian preparations for cytogenetic evaluation (i.e., each student will have to make satisfactory preparations).

May 19 - A.M. Introduction to mosquito reproduction including anatomy, mating behavior, blood meal ingestion, blood meal digestion, ovarian development, oviposition and refeeding. Relation of the gonotropic cycle to the estimation of survivorship and the dynamics of malaria transmission. Concept of vectorial capacity.

P.M. Mosquito dissection to learn (1) Christophers' classification of follicular maturation stages; (2) age grading using the tracheation and dilatation methods; (3) recognition of sacculate females whose dilatations have not yet contracted. Each student should dissect (1) unfed, recently fed, half-gravid and gravid females and classify the egg stages and (2) known nulliparous and

parous females to see changes in tracheal skeins and the formation of dilatations.

May 20 - A.M. Development of the malaria parasite in the mosquito after ingestion of gametes. Relationship of temperature and duration of gonotrophic cycle duration to the probability of transmission. Relationship of survivorship to establishing the "dangerous" portion of the population.

P.M. Dissection of infected and uninfected mosquitoes to observe oocysts on the gut and sporozoites in the salivary gland. Students should learn how to make permanent preparations for verification. Sporozoite concentration method should be introduced. Students should attempt to isolate sporozoites from mixtures of known infected and uninfected mosquitoes.

May 21 - A.M. Introduction to larval collection, survey and identification methods. Use of keys and introduction to the primary diagnostic characters.

P.M. Students should try to key out several species of larvae collected from the Hetauda area, including the vector species kept in the training center insectary. Students should also learn proper specimen preservation and slide preparation methods.

May 22 - A.M. Orientation to research schedule, collection techniques and record keeping. NMEO should go over in detail reporting methods to be sure each student understands how the data should be collected and recorded.

P.M. Students should be given blind tests to reveal their ability to determine the parity status of females of known reproductive history. The Hetauda laboratory staff should prepare a series of 10 females for each student to dissect. Senior NMEO staff will grade the tests and, if necessary, provide additional training.

4.3 Materials Required

The Research and Training Centre staff should prepare in advance the following mosquitoes for the proposed training course:

1. 50 gut- and gland-infected females for dissection and for study of the sporozoite concentration method

2. 150 uninfected females to mix with known infected females to evaluate the sporozoite concentration method

3. 60 known parous and 60 known nulliparous females for instruction and for dissection examination

5. COMMENTS ON JAPANESE ENCEPHALITIS VIRUS

Japanese encephalitis virus (JEV) is rapidly becoming a major public health concern in the terai region of Nepal.[1] At the request of NMEO and USAID, the following brief comments are provided to draw attention to the problem and outline possible strategies for surveillance and control.

5.1 Background and Rationale for Control

JEV is a flavivirus horizontally transmitted among avian hosts by several Culex mosquitoes. The virus also can be transmitted transovarially from mother to daughter by some Aedes and Culex species. Epizootics involving feral birds frequently spread to involve domestic swine and equines, with swine serving as a suitable amplifying host facilitating tangential transmission to man. Humans rarely produce sufficient viremias to infect mosquitoes, and man-to-man transmission is unlikely.

Temporal epidemics in Nepal have been associated with the monsoon when standing water in rice fields provides a myriad of breeding places for the vectors, including Culex tritaeniorhynchus, Cx. vishnui, Cx. pseudovishnui, Cx. fuscocephala and Cx. gelidus. Horizontal enzootic maintenance transmission may involve more ornithophagic species such as members of the Cx. bitaeniorhynchus group, while members of the Aedes subgenus Stegomyia have been shown to transmit the virus vertically.

As with most arboviruses, the ratio of inapparent to apparent cases is very high, i.e., relatively few infections produce clinical symptoms. In most vertebrates, apparent or inapparent infection produces long-lasting immunity which provides protection against re-infection. Thus, widespread epidemics may not be followed immediately by subsequent outbreaks. Virus maintenance requires large short-lived populations with a high recruitment rate of non-immunes.

Control strategies must incorporate a three-phase program of mosquito abatement, virus surveillance, and emergency control. A fourth alternative, human vaccination, is theoretically sound but would require a large initial foreign currency expenditure to purchase the vaccine from Japan, followed by the continued expenditure required for vaccination of children and immigrants into the endemic areas. A previous operational plan for the

control of JEV was presented by Pradhan.[4] The present brief outline iterates some researchers' conclusions and presents several additional ideas on virus surveillance and control.

5.2 Mosquito Abatement

Successful preventive mosquito abatement can keep vector mosquito populations below thresholds necessary for the epidemic spread of the virus to man. The following community self-help and NMEO input could reduce mosquito breeding in and around towns and cities.

1. Water management. All drains should be kept clean and freely flowing by city employees. Vegetation should be cleaned from ditches and permanent ponds on a routine basis. Once a week, inspectors (hired to augment NMEO staff) should check for mosquito larvae at all breeding places, including rice fields, within a one-mile radius of the town periphery. Rice fields with heavy breeding should be drained at two-week intervals to prevent emergence of adult mosquitoes.

2. Larvivorous fishes. Introduction of local larvivorous fishes into city ponds and other permanent breeding places should be encouraged. Health education workers should emphasize the importance of not eating fish placed for larval control.

3. Larvicides. Temporary breeding sites created during the monsoon period should be treated with oil or with insecticides (if available). The use of mosquito-specific compounds such as B.t.i. should be encouraged to protect the predatory insect population and prevent post-treatment rebound.

5.3 Surveillance

Three types of surveillance activities would seem useful for the terai area. At present, Nepal lacks the capability to isolate virus from tissue samples or from mosquitoes. Surveillance must be limited to decentralized serology testing[4] and monitoring of mosquito populations.

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[4]S. P. Pradhan. 1982. Proposed plan for the control of Japanese encephalitis in Nepal. mimeo 10 pp. Submitted to the Department of Health, Epidemiology Division.

1. Mosquito relative abundance. The marked rise in the abundance of suspect vector species can provide the first warning that an epidemic is pending. It also allows an evaluation of the efficacy of the mosquito abatement practices in 5.2 above. Sampling methodology should be sensitive for the suspect vector species and should include one or more of the following methods: (1) biting collections on bovid, equine or suid baits for two hours starting at sunset, (2) stable traps baited with calves or pigs, or (3) light traps. Large animal baits are preferable to humans because (1) the epidemic vector species are markedly zoophagic, and (2) it reduces the risk of infection to the collectors. Collections should be done at weekly intervals at several locations (number of collection stations should depend on the size of the human population at risk) and the data plotted graphically so that marked vector population increases are noticed immediately. Marked increases in mosquito abundance should be brought to the attention of the health authorities so they will be on the lookout for human cases.

2. Sentinels. Seroconversion rates in sentinel piglets should be monitored biweekly. Ten to 20 piglets interspersed throughout the town should be identified (tagged or tattooed) and pre-bled to ensure that they have had no previous exposure to JEV. The seroconversion of sentinels should be the trigger to initiate emergency control operations (see 5.4 below).

3. Human cases. Clinical diagnosis of JEV in man should be confirmed, when possible, by demonstrating a diagnostic rise in JEV antibody titres between acute and convalescent serum samples. High antibody titre alone, especially in acute sera, may mean a previous and not necessarily a current infection. Serological tests on both patient and sentinel sera should be done at zonal hospitals to allow rapid diagnoses.[4] Physicians should record and report all epidemiological information to a central agency which will collate the date to identify case clustering in time and space. Rapid data collation is essential if decisions on emergency control are to be made in a timely fashion.

5.4 Emergency Control

When indicated by surveillance data (one or all three in 5.3 above), emergency control operations should be initiated immediately. Spray equipment and insecticides should be purchased prior to the encephalitis season and made ready for

immediate deployment. Baseline data on mosquito susceptibility to adulticides should be monitored after each control episode. Staff should be trained in emergency control techniques, and should be immediately available for control operations.

The initial target of control operations should be to eliminate promptly the infected mosquito population from areas immediate and then peripheral to the human population at risk, using one or more of the following control methods:

1. Apply cold or thermal fog with truck-mounted equipment using malathion or another suitable insecticides at three- to seven-day intervals on at least three successive occasions.

2. Spray all animal shelters with residual insecticides. Houses most likely should not be sprayed because most residents sleep outdoors during the monsoon season due to the intense heat in the terai.

3. Encourage the population at risk to avoid mosquito bites through use of personal protection measures such as mosquito nets, insect coils, and house screening.

Note: Emergency measures are only useful if applied at the onset of the epidemic. By the time that large numbers of clinical human cases occur, it is usually too late for emergency control measures to protect the population at risk.

5.5 Research Needs

In addition to control-oriented activities, basic research should be conducted concurrently to gather ecological information necessary to refine forecasting and control methods. Some questions which should be addressed include:

1. What are the vector species in Nepal? Answering this question will require the development of an arbovirology unit consisting of virologists and entomologists. Laboratory procedures to isolate viruses from mosquitoes will be required.

2. What is the enzootic maintenance cycle in Nepal? This will require in-depth study by vertebrate zoologists, entomologists and virologists to study the cycling of the virus in the rice growing and forested areas of terai.

3. How does the virus persist between epidemics? Can the virus be found in Nepal throughout the year or is it reintroduced annually?

4. What is the best way to detect and arrest the spring amplification cycle of the virus?

5.6 Training Needs

Immediate training should be goal-oriented and conducted in Nepal by international specialists. Areas requiring immediate training include:

1. *Culex* mosquito identification, sampling, and control

2. Virological methods, especially serological diagnosis and virus isolation

3. Case management, reporting, and epidemiological assessment

APPENDIX A. PLAN FOR EXTENSIVE ENTOMOLOGICAL STUDIES
by S. L. Shrestha

Title	Intensive Entomological Studies in Hard Core Areas in Nepal
Duration	18 months (April 1987 to September 1988)
Principal Investigator	Mr. S. L. Shrestha
Co-investigator(s)	Mr. J. P. B. Shrestha Mr. J. D. Shrestha Mr. S. R. Shrestha
Collaboration Agencies	Vector Biology & Control Project Arlington, VA 22209, USA
Executing Organization	Nepal Malaria Eradication Organization National Headquarters, Kathmandu (Nepal)
Areas of Study	Sindhuli District of Central Region Kanchanpur District of Far-Western Region

Input

	HMG	USAID
Rs.	4,42,000	
US$	-	32,610

Endorsed by	Dr. M. B. Parajuli Chief Officer Nepal Malaria Eradication Organization

1. General Objective

To elucidate the causes of transmission.

2. Specific Objectives

a. To find out the causes for persistence of malaria transmission in the district Sindhuli of Central Region and the outbreak of the disease in the district Kanchanpur of Far-Western Region.

b. To elucidate if any other than the proven vector is responsible for the transmission of the disease.

c. To carry out longitudinal study covering at least one year period for the seasonal distribution of vector(s) as well as the behavior pattern in different seasons of the year.

d. To find out and recommend the suitable and economical control measures.

3. Background Information

Since the start of phased program of Malaria Eradication, the achievement was quite remarkable up to 1970-71 when only about 2,500 malaria cases were recorded for the whole country.

By the same year, 91 per cent of area were passed into the consolidation phase of MEP and the spraying was withdrawn. This state of affairs did not last very long and as in other countries of South-East Asia, the rise of incidence started from 1972 for various reasons including technical ones. The rise was almost double every year and reached the level of about 15,000 cases in the year 1975. In the year 1975, the in-depth study was carried out by the joint team of HMG/USAID/WHO and additional resources were made available to the program. As a result, the program was able to check the rising trend and maintain at the level of 12-16,000 cases annually.

Further deterioration started in 1984 when 29,388 cases were recorded. In the year 1985, an increase of an additional 44 percent was noted in 1985, reaching the absolute recorded case number of 42,321. One reason for this increase is it is appropriate to a decrease in spraying coverage. The investigation also led the investigators to suspect the transmission. The mention is that since the inception of anti-malaria activities, ecological changes have taken place covering vast areas of terai and inner-terai (valley between Mahabharat range in north and Churia siwalike range in south). There was extensive deforesta-

tion, extension of agricultural land, construction of irrigation channels and road, etc.

For the proposed study, two representative areas are chosen. One is in Ranibas and Mathillo Khutepani village of Sindhuli District in Central Region. The second is the Musepani/Gaugie villages and Badaipur/Pepariya villages of Kanchanpur District in the Far-Western Region. Both the study areas lie in a moderately receptive belt with An. fluviatilis as a proven vector.

4. The areas of Sindhuli and Kanchanpur are selected for the reason that the transmission could not be interrupted instead of all effort being made. Below are given the recorded number of cases in district, unit, locality, and also the study villages in the year 1983-85.

Area	1983		1984		1985	
District Sindhuli	279		738		1221	
1.1 Unit	203		432		809	
1.2 Locality (2)	22		249		439	
1.3 Study village	22		104		234	
District Kanchanpur	406		1969		6351	
2.1 Unit (II & IV)	305	67	740	737	2813	2321
2.2 Locality	4	1	42	48	72	245
2.3 Study village	3	0	20	9	27	104

5. Justification for the Proposed Study

It has already been noted that the transmission is still persisting in spite of insecticide spraying and regular surveillance mechanism in places of moderate receptivity. This may well be attributed to the occurrence of secondary vector which is refractory to the insecticide in use that have appeared as a result of ecological changes occurred in the area since the inception of anti-malaria activities 25 years before. There is also every possibility that the secondary vector that have appeared could be different depending upon ecological strata such as forested foot hills area and widely cleared area. Very recently in June 1985, An. maculatus along with An. fluviatilis have been incriminated from the foot hill area of terai where An. maculatus have never been proved before. The per man per night density of An. fluviatilis and An. maculatus were 2.0 and 2.66 respectively. Before this An. maculatus has only been proved as vector in high hill area at an elevation of 1980m above sea level.

If this proposed study is approved, this will also allow the program to study the bio-ecology of aquatic stages of An. maculatus along with An. fluviatilis which are otherwise known very little. Once the bio-ecology of aquatic stages are known, the other bio-environmental measures other than insecticide spraying also could be planned and implemented.

6. Methodology

The following techniques will be utilized to determine the parameters related to the calculation of vectorial capacity. Besides entomological data, the meteorological data and information regarding physical and biological conditions of the breeding places will be collected as given below.

6.1 Entomological

6.1.1 Indoor Hand Catch. Morning collection will be carried out in four human two mixed and two animal dwellings during early hours of 06:00-09:00. This will be repeated every fortnight.

6.1.2 Outdoor Catches. The collections from natural outdoor shelters will be made from 08:00-10:00 hours in each study area and repeated every fortnight. To supplement, four-pit-shelters (1.5m x 1.5x x 1.5m) with 30cm3 hole on each of the four side wall will be constructed and the collection will be made during each time of outdoor collections.

6.1.3 Night Catches. In each study area, mosquitoes alighted to the four human (two indoor and two outdoor or verrandah) will be collected. This will be from sun set to sun rise (i.e., dusk to dawn) that is approxi-mately from 18:30-05:30 depending upon the season of the year. The field supervisor will make supervision of each collector and also collect biting on animal. He will also collect mosquitoes that are resting close to the bait collectors. This also will be repeated every fortnight.

6.1.4 Larval Collection. Every fortnight, the larval collections will be carried out in each study area. The water bodies will be fixed for larval collection and so far as possible different types of breeding places will be included. The two collectors will make larval collections making 25 dips in breeding sites. Of the 25 dips, 5 dips in each of the four corner and 5 dips at the discretion of the collectors. While one collector makes the dip, the other collector will help him in transferring the larval specimen into the vials and record.

6.1.5 Determination of Age. The proven and suspected vector species will be dissected for ovary examination. The note will be made only of nulliparous and parous for different biotype.

6.1.6 Blood Meal Collection. The blood meal will be collected from all specimen of anopheline mosquito which are found resting outdoor and also about 100 for each species (proven and suspected vectors species only) from different type of dwellings every season. The blood meals will be identified by gel diffusion techniques at Kathmandu.

6.1.7 Gland Dissection and Examination for Sporozoites. All the proven and suspected (on the basis of Anthropophily or species having high degree of contact with man) vector species will be dissected for the examination of sporozoites in their glands and record.

6.1.8 Susceptibility Test. Test like susceptibility will be carried out every season. Similarly irritability test to determine the irritability to different insecticides will be performed at least once during the study period.

6.1.9 Meteorological Data. The meteorological data will be obtained from the irrigation depart-ment which is well equipped in the case of the District Kanchanpur. For the District Sindhuli, the team should be equipped for the same.

6.2 Epidemiological

The surveillance activities which exist at present will be continued. This includes active case detection by malaria staff making house-to-house visits at an interval of

one month. Passive case detection through voluntary collaborators, in the malaria offices, and by the malaria clinics in the District Hospitals will also be continued. The administration of presumptive treatment at the time of collection of blood (600 mg chloroquine: adult dose) will also be continued. All the confirmed cases will be radically treated with 15 mg of chloroquine given in three days and 75 mg of primaquine in five days.

The epidemiological investigation of all confirmed malaria cases will be carried out with a view toward determination of species of parasites, and time and likely place of infection. All the cases shall also be followed up for a 12-month period during which slides will be taken every month.

6.3 Spraying

The study villages shall not be sprayed for the entire period of study.

7. Collaboration

This study will be implemented in Nepal. A consultant from the VBC Project, USA, shall pay a visit for about one month before the start of the project. The same consultant shall also make a visit once during the study period and at the end.

The local malaria advisor, USAID, will also participate in the study as requested by the principal investigator.

8. Reporting and Recording

All the data related to the study will be recorded. The proforma of the forms to be filled in for reporting and recording will be done prior to the start of the study.

The epidemiological data will be obtained from the District Malaria Office and verified by the principal investigator in the field or other upon request by principal investigator.

9. Personnel and Percentage of Time Devoted to the Project Work

Principal investigator	1 (20%)
Co-investigators	3 (25%)
Chief, Entomology Section, NHQ	1 (30%)
Local consultant	1 (10%)
Malaria assistants One for each team and one to help PI	3 (100%)
Malaria assistants	4 (100%)
Insect collectors	8 (100%)
Laboratory aide	2 (100%)
Driver	2 (100%)

10. Budget

Code No.		Estimated Budget Required
1	Salary	2,25,000
2	Allowance	50,000
3	TA/DA	90,000
4.1	Services	5,000
4.2	Services/other	8,000
5	Rent	6,000
6	Repair/Maintenance	8,000
7.1	Office Equipments	5,000
7.1.1	Fuel/Vehicle	25,000
7.3.2	Fuel/Other	8,000
7.5	Other Materials	10,000
8.3	Medicines	2,000
		Rs. 4,42,000

The budget amounting to Rs. 4,42,000 shall be borne by HMG, NMEO from its regular budget allocation.

In addition, USAID will bear the expenses required for the consultant to be provided from VBC, Washington and the equipment and supplies amounting to US $32,610 as per list attached.

List of Supplies

1.	Aspirator (plexi glass, 1 feet long, external diameter ½ inch)	6 doz.	US $ 75.00
2.	Tygon tube (internal diameter ½ inch)	100 ft.	US $ 75.00
3.	Test tube (pyrex. no. 9800)	24 doz.	US $ 150.00
4.	Mechanical aspirator with battery (battery operator) (hand battery vacuum cleaner, Mansherr's Machine Works, Toms Rivers, NJ 08753, USA)	12 pcs.	US $ 150.00
5.	Centrifuge - 1000 RPM (portable)	1 pc.	US $ 200.00
6.	Centrifuge machine (220 volts) (10,000 RPM)	1 pc.	US $ 5,000.00
7.	Tissue grinder AA (Thomas Catalogue No. 3431-E12)	2 pcs.	US $ 300.00
8.	Honda Trial 110 cc (trial)	4 pcs.	US $10,000.00
9.	Microscope compounds, binocular (American optical)	4 pcs.	US $ 2,400.00
10.	Stereoscopic microscope, binocular (American optical)	2 pcs.	US $ 600.00
11.	Camp cot	8 pcs.	NC 8,000.00
12.	Gumboots	8 pcs.	NC 1,600.00
13.	Water filter - 10 lits. capacity	2 pcs.	NC 2,000.00
14.	Sling psychometer (Thomas Catalogue No. 6067-N10)	2 pcs.	US $ 60.00
15.	Incubator (Thomas Catalogue No. 6119-E15)	1 pc.	US $ 2,000.00
		TOTAL	US $32,610.00

APPENDIX B. ITINERARY FOR WKR TRIP TO NEPAL MARCH - APRIL 1987

March 23	Discussed purpose of visit and consultant role with USAID/Kathmandu. Discussed research protocols for Central Region with NMEO headquarters staff.
March 24-25	Discussed research protocols and field sampling with NMEO staff at Tanibas and Kutepani study villages in Sindhuli District. Assisted with mosquito collection and dissections.
March 26	Visited Bhiman Unit Office #2, District Sindhuli, to obtain sketch maps of the study villages and malaria case data for 1987. Traveled from Sindhuli study area to Kathmandu.
March 27	Met with NMEO Headquarters staff to discuss trip to Central Region and outline activities for trip to the Far Western Region.
March 28-29	Traveled from Kathmandu to Dhangardi.
March 30-31	Discussed research protocols and field sampling with NMEO staff at Badaipur and Musepani Villages, Kanchanpur District. Observed field collections and dissection for sporozoites.
April 1	Debriefed NMEO staff at Regional Office. Traveled from Dhangardi to Kathmandu.
April 2-4	Prepared protocol review, research working plan and outline of training program for intermediate staff at Hetauda.
April 5	Met with Chief of NMEO to discuss research protocol objectives and relation to malaria control in Nepal. Comments on JEV added to report at request of NMEO and USAID.
April 6-7	Reviewed report draft with USAID and prepared final copy for local distribution.
April 8	Debriefed NMEO and USAID.

APPENDIX C. PERSONS CONTACTED

Kathmandu

Dr. David H. Calder, Chief, Health and Family Planning, USAID

Ms. Barbara J. Spaid, Assistant Health Development Officer, USAID

Mr. Joseph L. Anderson, Population Specialist, USAID

Mr. Shreedhar P. Pradhan, Malaria Advisor, USAID

Dr. Kallyan M. Acharya-Dixit, Chief Officer, NMEO

Dr. R. R. Arora, WHO consultant to NMEO

Mr. Jitendra P. B. Shrestha, Deputy Chief Officer, NMEO

Dr. Manas K. Benarjee, Epidemiology Section, NMEO

Mr. Rameshar P. Sharma, Section Chief, Operations, NMEO

Mr. Rhiddi P. Vaidya, Regional Malaria Officer, Western Region, NMEO

Central Region

Mr. Janak D. Shrestha, Regional Malaria Officer, Central Region, NMEO

Mr. Raghu N. Sing, Unit in Charge, Bhiman Unit Office, NMEO

Far Western Region

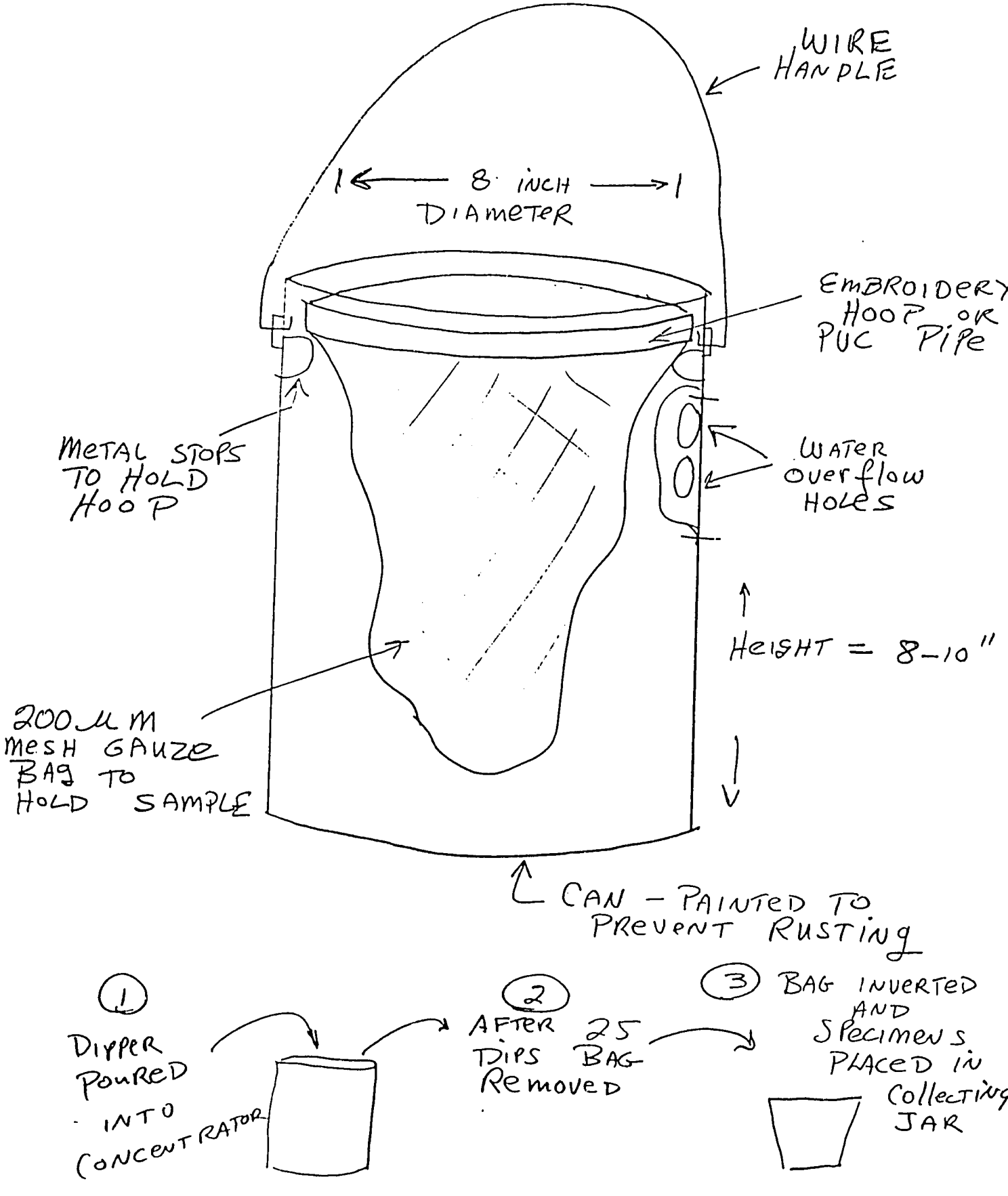
Mr. Shambu R. Shrestha, Regional Malaria Officer, Far Western Region, NMEO

Mr. Govind P. Shrestha, Malaria Officer, Kailali District, NMEO

Mr. Tekendra B. Swar, Malaria Officer, Kanchanpur District, NMEO

Mr. Ramananda R. Mandal, Entomologist, Far Western Region, NMEO

APPENDIX D. LARVAL SAMPLE CONCENTRATOR



APPENDIX E. TENTATIVE SCHEDULE OF FIELD VISITS BY NMEO, USAID AND VBC STAFF TO STUDY AREAS*

Year	Month	VBC	USAID	NHQ NMEO
1987	APR	C+W	W+C	
	MAY			
	JUN		W	W+C
	JUL			
	AUG		C	W
	SEP			
	OCT			
	NOV		W	C
	DEC			
1988	JAN			
	FEB			
	MAR	C+W	C+W	W+C
	APR			
	MAY		W	C
	JUN			
	JUL			
	AUG		C	W
	SEP			
	OCT	W+C?	W+C	W+C

*W = Far Western Regions, C = Central Regions. The USAID advisor and NMEO staff will accompany the VBC consultant to the field to discuss modifications in research design during year 2. Field teams will be under the direct supervision of the regional malaria officers.