The value of malaria diagnosis: a preliminary study in the Terai districts of Nepal

Final Report

(June 1999 to 2001)

To

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Annexes:

Abbreviations

ACD	Active case detection
API	Annual parasite index
CNS	Central nervous system
DNA	Deoxyribo nucleic acid
DHO	District Health Office
ETF	Early treatment failure
EDCD	Epidemiology and Disease Control Division
ELISA	Enzyme Linked Immunosorbent assay
EPR	Estimated positive rate
EDPT	Early detection and prompt treatment
FNR	False negative rate
FPR	False positive rate
GIS	Geographical information system
HRP	Histidine rich protein
HP	Health Post
HMG	His Majesty's Government
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ICT	Immunochromatographic test
IFAT	Indirect fluorescent assay test
ITDRC	Infectious and Tropical Disease Research Centre
JE	Japanese encephalitis
JICA	Japan International Cooperation Agency
JMA	Japan Medical Association
LTF	Late treatment failure
LSTM	Liverpool School of Tropical Medicine
NHRC	Nepal Health Research Council
PCR	Polymerase chain reaction
PCD	Passive case detection
pLDH	Parasite lactase dehydrogenas
PV	Plasmodium vivax
PF	Plasmodium falciparum
QC	Quality control
RCS	Research capability strengthening
SHP	Sub-Health Post
TDR	Tropical Disease Research
TUTH	Tribhuvan University Teaching Hospital
TPR	True positive rate
TS	Treatment success
UP	Uttar Pradesh (India)
UNDP	United Nation Development Project
VBDRTC	Vector Borne Disease Research and Training Centre
VDC	Village Development Committee
WHO	World Health Organisation

Summary of report

As a result of a combination of events, a situation has developed in Nepal where cerebral malaria is misdiagnosed and, therefore, not treated. This was demonstrated in a hospital admission study conducted in three districts in Southern Nepal (Dhanusha, Banke and Kanchanpur), where microscopical blood examination was routinely performed on all daytime in-patients, regardless of the clinical presentation; these data were retrospectively compared to the admission category each of these patients had been classified as. In the 5,220 patients included in the study, there was a clear clustering of the 579 malaria cases in the admission categories describing systemic/neurological symptomatology (including convulsions, meningitis, encephalitis); in particular, of the 142 cases of *P. falciparum* observed, 27 were clustered in the "encephalitis/CNS manifestations" group. An investigation of the reason for this clustering showed that an inappropriate diagnostic algorithm for febrile coma was used by medical officers, which lead to an over-diagnosis of Japanese Encephalitis at the expense of cerebral malaria. Since the number of malaria cases due to *Plasmodium falciparum* is on the increase in Nepal, together with an increasing problem of drug resistance, the misdiagnosis of severe cases is likely to become a significant cause of mortality.

In order to correct the problem and save lives, it will be necessary to abandon the inappropriate algorithm, retrain medical officers to recognise a clinical entity they are unaware of, review the need for specialist malaria microscopists if their function is only to collect malaria prevalence data and, failing this, make the clinical staff of district hospitals aware of the need for considering emergency malaria diagnosis and treatment as a routine protocol in the management of any febrile neurological problem.

The study was performed over a period of 16 months and, in view of the results obtained (which confirmed and expanded the original hypothesis), it became clear that additional information on malaria in Southern Nepal was needed. It was necessary to check the quality of microscopy, examine whether new antigen detection tests could feasibly be used in Nepal, perform preliminary studies to use PCR for diagnosis and quality control, set up methods for

looking at drug resistance both for *P. falciparum* and *P. vivax*, investigate the role of imported malaria.

These studies were of general benefit to the capacity strengthening of our research centre, which is currently the only laboratory in the country where *P. falciparum* can be grown *in vitro* and where molecular studies of drug resistance can be performed. The centre could in future, and in collaboration, with the National Malaria Control Programme, provide the necessary research input to malaria control programmes.

1. Report and research activities

1.1. Introduction:

Malaria is estimated to be the cause of between 1 and 3 million deaths world-wide each year, and the situation is worsening in many parts of the developing world as a result the development of resistance to drugs and insecticides by infectious agents. The prevalence of the disease is generally related to environmental factors or seasonal weather trends and to human activities such as migration, settlement, agriculture and exploitation of natural resources. Because of this broad spectrum of contributing factors, it has been suggested that malaria control would benefit from a more inter-sectoral effort, although it has never been clear how such an initiative could be implemented. In Nepal, nearly 13 million people (64% of the population) are at risk of contracting malaria (EDCD, 1995). Five vector species *Anopheles (An) fluviatilis, An. minimus, An. maculatus, An. willmori* and *An. annularis* have been found to be responsible for malaria transmission in Nepal (Malaria annual report, 2001).

In the past, the Nepal National Malaria Control Programme consisted of vertical programmes, with a centralised structure and a staff of well-trained specialists who dealt with all the different aspects of malaria control (diagnosis, epidemiology, vector control, treatment). Such a structure was particularly well developed and effective throughout the Indian Sub-Continent, including in Nepal. In the early 1980s, such costly structures were considered inappropriate in poor countries, and World Bank pressure led to the integration of malaria control into primary health care programmes. However, the switch from one system to the other was not well-thought-out,

and, it has lead to the existence of a hybrid structure which lacks the resources and motivation of a vertical structure and becomes increasingly ineffective. The situation is rapidly changing, with a switch from a situation where infections with the mild *Plasmodium vivax* are replaced by infections with the far more life-threatening *P. falciparum*. In Nepal, for example, over 20% of new cases are now believed to be *Falciparum* malaria. The statistics of the National Malaria Control Programme of Nepal indicate that annual parasite index (API) is approximately 20,000 new cases each year (EDCD, 1998). Because of the current state of case reporting, this figure in almost certainly an underestimate. In fact, there are no official reports on mortality attributable to *falciparum* malaria (EDCD, 1999). A previous study performed in Southern Nepal of Janakpur (Sherchand *et al.*,1995), involving hospital-based active case detection, as well as detailed village surveys and verbal autopsy questionnaires, has confirmed the impression that the absence of severe morbidity and mortality due to *P. falciparum* in Nepal was probably untrue and that a likely explanation for the lack of reporting was inappropriate clinical diagnosis (medical officers being unaware of the clinical condition and confusing the symptoms of severe *Falciparum* malaria with other syndromes, e.g. confusion between cerebral malaria and meningitis or *Japanese B encephalitis* and consequent over-reporting of the latter). According to the report of Vector Borne Disease Research and Training Centre (VBDRTC), the overall malaria situation in 1997 showed 8957 positive cases from 160293 slides collected; two deaths were reported. *Plasmodium falciparum* cases (1150 cases) were highest in Western region (63%), followed by Far Western region (14%), East region (12%), Central region (8%) and Midwestern region (2%).

Most of the reporting is based on passive case detection (PCD) and active case detection (ACD) is non-existent. PCD volunteers are no longer in place in many areas, a situation which should be revised and strengthened to improve early detection and treatment for malaria cases. Laboratory services in District Health Office (DHO) and Primary Health Centres (PHC) are established but in health post and sub-health post (HP/ SHP) no laboratory services are available. Hence, most patients having fever in Terai and inner Terai are empirically diagnosed as malaria without microscopic confirmation of the presence of malaria parasites in the blood sample and such patients are treated with antipyretics or with chloroquine. Hospital admission cases are grossly underdiagnosed for malaria. Similarly, severe or cerebral malaria tend to

misdiagnose in the hospitals as meningitis or encephalitis (usually as JE) as a result of an inappropriate diagnostic algorithm for febrile coma, which has been in use in Nepal for many years.

Anti-malarial drug resistance problem is an obstacle for prevention and treatment of severe *Falciparum* malaria. It will be important to monitor drug sensitivity to document the extent and distribution of resistance. A range of new methodologies is available which make the diagnosis of malaria easier to perform and more reliable; some of these techniques were tested in this study.

Although malaria can be cured, the proper management of this disease malaria depends on the accurate diagnosis and the efficiency of the medical facilities. In most of the areas of Nepal, health workers are often unable to cope with the burden of diagnosis and appropriate treatment. Lack of laboratory equipment, drugs and skilled manpower as well as the presence of drug resistant parasites, limit the possibilities of handling malaria cases, and treatment decisions in many malaria endemic areas and hospitals.

2. The study objectives:

To test the hypothesis that improved malaria diagnosis at the hospital level, rather than at the health post level, could significantly improve the management of severe *Falciparum* malaria and thus substantially increase the value of a National Malaria Control Programme.

3. Study areas

The study was conducted initially in two districts (Dhanusha and Banke) of the Terai region between 1999 and 2000. Subsequently a third district (Kanchanpur) was included with Dhanusha district up to the period of 2001. The description of the study districts are presented in Annexe 1 and the location of study areas are shown in Figure 1.

4. Study design:

4. 1. Methodology:

The study was designed to gather primary data regarding an important issue of malaria control: the diagnosis and management of severe *Falciparum* malaria. The study explored a possible ways of dealing with the misdiagnosis of severe malaria in Nepal, within the existing policy of a malaria control programme integrated within primary health care. In this pilot study, 6 trained malaria technicians were assigned for duration of one year in two district hospitals (Dhanusha and Banke). They were given one week further training to ensure confidence in the recognition of different species of malaria parasites, sample collection, transportation and methods of antigen detection. They were also trained in the use of questionnaires and its administration. One day training was given in the form of question and answer sessions. Any ambiguities in the questionnaire were discussed until a consensus was reached as to the exact meaning of the term used. The researcher attempted to find interviewers with no direct links to the health sectors, as this could have biased people's opinions. They were instructed to keep all the data and information with confidential.

In the hospitals, interviewers were involved collecting systematic information such as name, age, sex, address, current situation of clinical history, past history of treatment, knowledge, attitude and practices. Clinical fever and abnormality were also observed from patient and their status of day in-patient admissions, after the primary diagnosis has been made. (i.e. every patient admitted during the day time were included in the study regardless of symptoms or primary diagnosis; night-time admissions were not included). Detailed information of the geographical origin of all hospital admissions were recorded and the origin of malaria cases were mapped against this background. In addition, the level of misdiagnosis was assessed (i.e. by defining which major clinical syndromes are more often associated with *Falciparum* infection). Finally, we worked closely with clinical staff to improve clinical identification of *Falciparum* malaria by local medical officers and hospital doctors, and to evaluate the capability of the hospital to offer treatment for severe *Falciparum* malaria.

In addition, two separate study were conducted in two malaria endemic districts of Nepal to compare the performance of OptiMAL, an immunochromatographic antigen detection assay for

the diagnosis of malaria using parasite lactate dehydrogenase (pLDH), against standard *microscopy* in patients with suspected malaria. The second study was carried out between August and December, 2001 among adult population of Terai from suspected malaria patients with or without travel (cross-border) history.

4.2. Sample size and sampling methods:

The study included over 5000 blood samples from hospital patients and community people. Of the 4039 blood samples from the hospital admitted patients, 1858 samples were from Dhanusha district and 2181 from Banke district. Blood smears (thick and thin) were made, dried and stained with Giemsa stain. Smears were examined for malaria parasites under a microscope in 100 fields using a x 1000 magnification. Sera were separated and stored at -20 degrees celcius until the test performed. Those patients who refused to give blood were not included.

In vitro cultivation, immuno-diagnostic tests such as ELISA, IFAT, antigen detection test and PCR were carried out in Tribhuvan University Institute of Medicine, Health Research Laboratory.

A prospective study using a standard WHO protocol for *in vivo* drug efficacy testing was not a part of the original project objectives, but was included in answer to a request from the Ministry of Health. The study was carried out during a period of 12 weeks between June and August 2000. 58 patients confirmed by a positive blood slide for *P. falciparum* who attended malaria clinic, who were eligible for the study and who also agreed to participate were enrolled to detect the level of the *in vivo* chloroquine efficacy test, in order to assess the need for change in the management and treatment of uncomplicated malaria.

Two additional studies were performed between August 2000 and December 2001, with a sample size of 618 (180 samples from study 1; and 438 samples from study 2). In study one we carried a rapid immunochromatographic OptiMAL assay for detection of *P. vivax* and *P. falciparum* malaria from two endemic districts of Nepal and, in study 2 we studied on Malaria infection: a preliminary study among Terai People who crossed or never crossed Southern Border of Nepal.

4.3. Data analysis

The hospital admission study was performed in Dhanusha District Hospital (Janakpur), Nepalganj District Hospital (Banke) and Kanchanpur District Hospital. In all studies, the following standard admission categories were used: fever/headaque; chill/rigor; vomiting; convulsions; gastroenteritis/diarrhoea; hepatitis/jaundice; typhoid; respiratory infection; encephalitis/CNS manifestation; meningitis; measles; septicaemia; tuberculosis; genito-urinary infection; kala azar suspected; malnutrition; cancer suspected; eye infection; skin infection; others (including diabetes mellitus, hypertension, surgical cases, tetanus and snake bite). These admission categories are based on those normally used by all hospitals in Nepal for reporting purposes.

The relative percentage of slide positivity was recorded for each admission category and compared to the expected rate of positivity based on the average positivity rate for all the hospital admissions over the period of study. Any admission category for which there is a substantial increase of slide positivity suggests that malaria is either the real cause of the symptomatology or an aggravating factor.

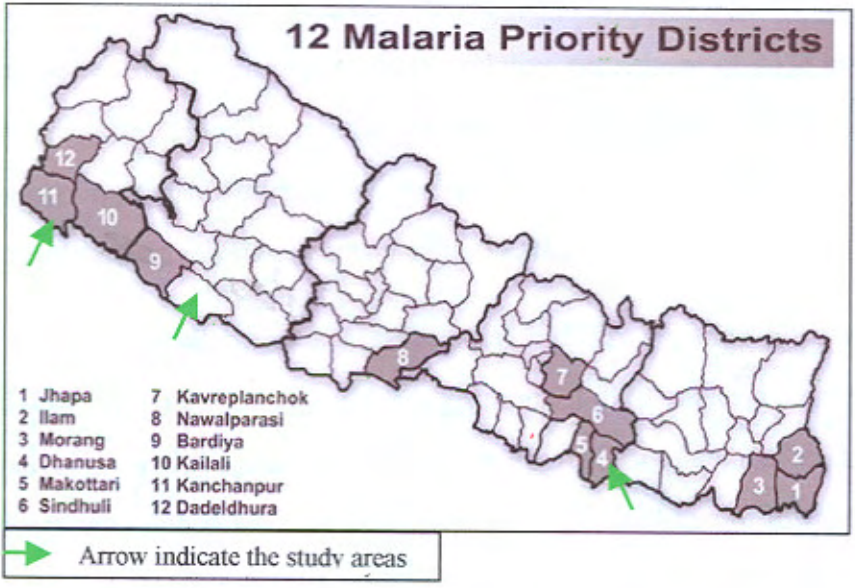
The relative frequency of *P. falciparum* infection compared to other species (*P. vivax* and *P. malariae*) was recorded. Any admission category in which there is a substantial increase of *P. falciparum* frequency suggests an increased risk of severe malaria.

Analysis was performed separately for every hospital since the variable prevalence rates precluded from an overall analysis.

4.4. Ethical consideration:

This study received an ethical clearance letter from Nepal Health Research Council (NHRC).

Figure 1 Map of Nepal: showing the location of study areas



5. Results:

5.1. Results of hospital admission study:

In any study of malaria, it is always difficult to define what contribution *Plasmodium* infection actually has in disease, since it is possible in an endemic area to carry parasites and be admitted to hospital for other causes.

The nature of the study performed, where the only contribution made by the investigators is a random blood film made on all day-admissions who agree to be part of the study (the admission diagnosis having been pre-determined at the health post which referred the patient), the analysis of data can be based i) on the study of the relative percentage of slide positive for each of the admission categories; ii) on the relative frequency of *P. falciparum* infection compared to other species (assuming that *P. falciparum* is responsible for the more severe forms of malaria); and iii) on a quantitative analysis of parasitaemia (*i.e.* the determination of a 'clinical threshold').

Any category in which there is a substantial increase of slide positivity compared to the expected rate of positivity, would suggest either that malaria is the real cause for the symptomatology or that malaria represents an aggravating factor of that particular syndrome. Any category in which *P. falciparum* is more frequently observed than other species would be suggestive of increased risk of severe malaria. The issue of clinical threshold has not been considered in the present study. The difficulty at this stage of the investigation and with the relatively small number of patients included in the study so far is to define what a 'substantial increase' in frequency actually is.

Four hospital admission studies were performed: two in Dhanusha, one in Banke and one in Kanchanpur. A total of 5,220 patients were included in the study. Table 1 lists the admission categories in which slide positivity was substantially increased and Table 2 lists the admission categories in which *P. falciparum* frequency was substantially increased. Despite the variation from one study to the other, a cluster of admission categories with systemic/neurological symptomatology (including convulsions, meningitis, encephalitis) showed a significant increase both in the percentage of slide positivity and the relative frequency of *P. falciparum* infection. Interestingly, the fever/headache admission category which scores high as a group associated

with malaria infection (Table 1) but does not score as a group suggestive of *P. falciparum* infection, which validates the analytic approach since fever is clearly not a distinguishing feature between Vivax and Falciparum paroxysms. Other admission categories had a slide positivity and relative *P. falciparum* prevalence similar to or below the hospital average; for example, the "gastro-enteritis" admission category, which was numerically the largest in all studies had a 14.7% slide positivity in Dhanusha (compared to a hospital average of 19.1%) and 1.1% in Banke (compared to a hospital average of 7.4%).

Table 1.

Name of the study areas	Number of patients recruited	Average slide positivity for hospital	Admission categories with increased slide positivity
Dhanusha (Study 1)	743	3.0%	**Meningitis** (18.2%) **Fever/headache** (13.6%) **Chill/rigor** (13.6%) **Encephalitis** (13.6%) Others (9.1%)
Dhanusha (Study 2)	1,858	19.1 %	**Convulsions** (85,7%) **Fever/headache** (83%) **Chill/rigor** (40%) **Encephalitis** (35,2%) Malnutrition (27.4%)
Banke	2,181	7.4%	**Vomiting** (33.3%) **Convulsions** (27.1%) **Fever/headache** (26.6%) **Respiratory disease** (18.2%) **Chill/rigor** (17.2%) Malnutrition (13.3%) Encephalitis (9.4%) Hepatitis/jaundice (9.1%) Meningitis (9.0%)
Kanchanpur	438	9.6%	**Encephalitis** (25%) Fever/headache (15%) Chills/rigor (15%) Vomiting (15%) Diarrhoea (15%)

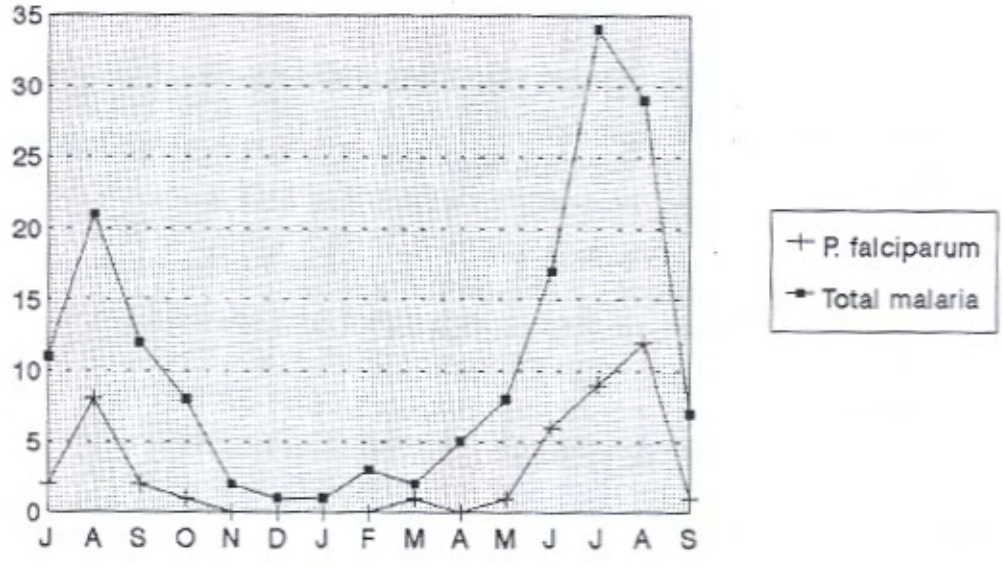
Table 2.

Name of the study areas	Number of patients Recruited	Average *P. falciparum* prevalence	Admission categories with increased Pf prevalence
Dhanusha (Study 1)	743	55%	**Convulsions** (100%) **Meningitis** (75%) Chill/rigor (66%) Encephalitis (66%)
Dhanusha (Study 2)	1,858	25%	**Encephalitis** (44%) **Convulsions** (40%) Vomiting (37.5%) Hepatitis (33%) Kala azar suspected (33%) Malnutrition (28,6%)
Banke	2,181	27%	**Encephalitis** (86,6%) **Convulsions** (38.5%) Malnutrition (37.5%) Vomiting (33%) Typhoid (33%)
Kanchanpur	438	43%	**Encephalitis** (100%) **Vomiting** (61%)

5.2. Month-wise distribution of malaria infection:

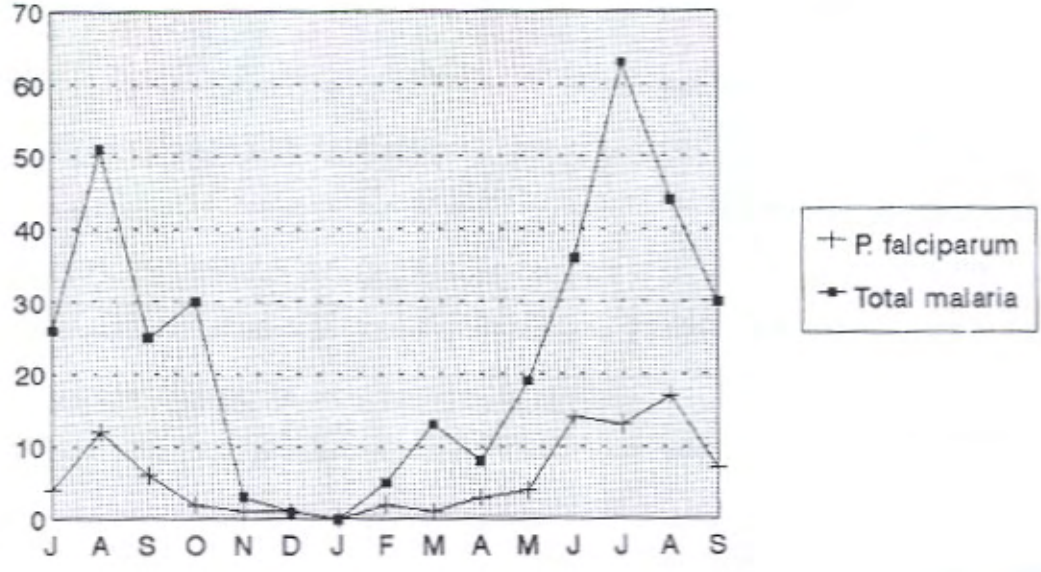
The higher distribution of malaria parasite infection in the study districts was during summer and rainy seasons- June, July and August as shown in Figure 2 and 3. The prevalence decreased during the winter, although there are some cases, which were detected in early winter in October and September, and pre-rainy seasons in March, April and May.

Figure 2. Monthwise distribution of *P. falciparum* vs total malaria (Dhanusha district)



(15th July, 1999 to 14th Sept., 2000)

Figure 3. Monthwise distribution of *P. falciparum* vs total malaria (Banke district)



(15th July, 1999 to 14th Sept., 2000)

6. Sero-diagnosis of malaria in relation to *Japanese encephalitis*:

The study was carried out in July, August and September 2000, during an epidemic of *Japanese encephalitis*. A total of 552 serum samples were collected from the hospital admitted patients who were clinically diagnosed as Japanese encephalitis. 152 sera were from Dhanusha and 400 serum samples were from Banke. During the blood collection from these selected cases both thick and thin smear were also prepared and stained with standard Giemsa stain. Smears were examined for malaria parasites under a microscope in 100 fields using a x 1000 magnification. Parasite density was calculated by counting the number of parasites against 200 leucocytes and multiplied by 40 to obtain a density per cu mm. Parasite rates as well as positive parasites density indices (PPDI) were calculated using the scale defined by Bruce Chwatt (1988).

In order to perform the immunological tests, IgG and IgM ELISA capture tests were performed separately for malaria and *Japanese encephalitis*. In 102 patients with highly suspicious symptoms of severe malaria, including fever, chills, sweats, headache and CNS manifestation finger prick blood was collected in heparinised tubes. Commercial Dipstick rapid test for detection of *P. falciparum* histidine rich protein -2 (Pf HRP-2) were perfomed on the spot to confirm the presence of malaria infection.

6.1. Results:

Out of 552 blood samples tested 316 (57.2%) were significant antibody titre against *P. falcipaum* and 201 (36.4%) had a JE-IgM antibody titre (Figure 6). Eightytwo (14.8%) of the thick smear were positive for malaria. Of the 82 positive malaria, 34 (6.1%) were positive for *P. falciparum* and 48 (8.7%) were *P. vivax*. This percentage of *P. falciparum* infection was significantly higher than the average in the local population (12.5%). Of the 102 cases with suspected *P. falciparum* malaria, 36 were positive by dipstick test for *falciparum* malaria, 34 of which were later found microscopy positive. Two cases which were not detected by microscopical examination, but positive by dipstick test, both showed significant antibody titre against *P. falciparum* antigen by ELISA. After one weak, previously negative blood smear were re-tested and one case positive with clear *falciparum* gametocytes.

The results obtained confirm earlier, similar studies performed by our group (Sherchand *et al.*, 1998) in other parts of Nepal and shows that while JE is clearly a cause of encephalitis in

Nepal, there is an overlap with cerebral malaria. Further work is needed to elucidate better ways of avoiding the misdiagnosis of cerebral malaria because, unlike JE, cerebral malaria is actually treatable in a large number of cases, if detected early.

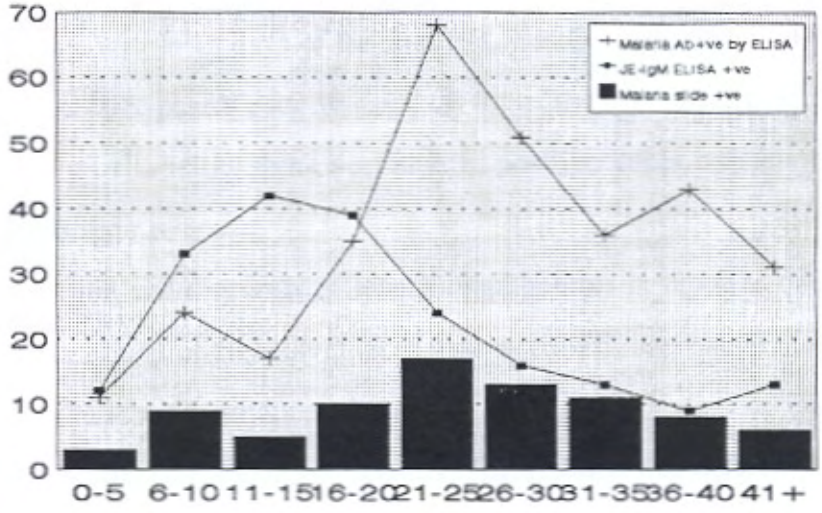
6.2. Parasite density:

The highest parasitic density (above 1600) were found in *P falciparum* cases and low density (below 100) were observed in *P. vivax* malaria patients, however, there was no significant difference between parasite density and the infection from these two parasites ($P > 0.05$).

Parasite density Index: The crude parasite density rate or index is = 2.85 (234/82)

Density class	Number	x class
1= < 100/ µl	19	x 1= 19
2= 101- 200	23	x 2= 46
3= 201- 400	14	x 3= 42
4= 401- 800	9	x 4= 36
5= 801-1600	11	x 5= 55
6= > 1600	6	x 6= 36
Total	82	234

Figure 4. The distribution of malaria, malaria antibodies and JE-IgM ELISA units by age groups (Banke and Dhanusha districts of Nepal)



N=552 (MP+ve=82; Malaria-ELISA +ve= 316; JE-ELISA +ve= 201)

7. Preliminary study on molecular approaches among people of asymptomatic *Plasmodium vivax* malaria:

In Nepal, Tharu communities of Southern Terai have been described as having natural resistance to malaria (Sherchand *et al.,* 1995; 1996). Although individual repeatedly infected with *P. vivax* may have attenuated symptoms, absence of symptoms has never been described in endemic Terai region of Nepal where *P. vivax* malaria is predominant. In the population of Kanchanpur-village a few individuals have shown to have blood parasites in absence of clinical symptoms. This suggests that a fraction of the native population may be resistant to malaria and may serve as reservoir of the infection.

To test this hypothesis we have studied a native population, adding PCR -amplification of ribosomal DNA to clinical examination and blood-smear microscopy for the diagnosis of vivax malaria. 76 inhabitants of Kanchanpur village were followed up from June, 2000 to August, 2000. 13 cases of *P. vivax* and 3 cases of *P. falciparum* were diagnosed. All cases had malaria symptoms with positive blood smears and positive PCR. The majority of this population had lived for more than two year and were less than 20 years old. We examined and confirmed 22 individuals between 16 and 40 who were PCR- positive for *P. vivax,* but without symptoms. 15 were kept under medical supervision for one month. Nine had positive blood smears with very low density of parasitaemia. No patient developed malaria symptoms in next one month follow-up. Within 15 days of the first test, 9 individuals became PCR- negative, but six patients remained PCR-positive for 30 days. Of these, three also had positive blood smears. It is difficult to mention whether these persistently positive cases correspond to long-lasting infections, reinfections, or relapses.

Precise detection and diagnosis of Plasmodium species in blood is of major importance in determining the treatment of the individual as well as control approaches for the community. Although several laboratory procedures already exist for identification of malaria species, the recent technology can provide additional sensitive information not offered by other methods. PCR-amplification of ribosomal DNA is a very sensitive and species-specific diagnostic method for malaria that detects only living blood parasites. These results open a number of questions not only about malaria control programmes in the study areas, where native

populations may act as reservoir of malaria, but also about mechanisms involved in the acquired immunity to *P. vivax*. Hence, to obtain definite information and substantial evidence of symptomless vivax malaria infection further comprehensive study is require in other endemic areas of Nepal.

8. *In vivo* drug susceptibility test:

8.1. Chloroquine sensitivity test using *in vivo* drug efficacy method:

Though this study is not a part of current project objectives, but due to on request of concern authority of Ministry of Health, Nepal insisted that the work be included.

For many years, choloroquine has been the first line drug for treatment of uncomplicated malaria in Southern Terai. Its main advantage of being effective, usually well tolerated and relatively cheap, making treatment of high numbers of cases affordable. In many parts of Terai region, access to chloroquine is easy, as national policy encourages its distribution through the national and health care system, village health workers and health post.

The objective of the current study was to detect the level of the *in vivo* chloroquine efficacy in *falciparum* malaria infections, in order to assess whether there is a justifiable need for change in the management and treatment of uncomplicated malaria.

A prospective descriptive study using standard protocol of WHO of *in vivo* drug efficacy test (WHO, 1996) were carried out during a period of 12 weeks between June and August 2000. 58 patients (25 patients from Banke and 33 patients from Dhanusha) confirmed by a positive blood slide for *P. falciparum* who agreed to participate were included.

8.2. Field procedures:

During the study period the patient's clinical history and body temperature were recorded on day 0, 1, 2, 3, 7 and 14. Fever was regarded to be present at temperature above 98.5 F.

Microscopic confirmation were done using both thick and thin smear with standard Giemsa staining method. Patients who had previous a history of antimalaria treatment within 7 days prior to the study period were excluded from the study.

8.3. Treatment: Patients included in this study were administered oral treatment with chloroquine. The intake of antimalarial drugs by malaria patients was strictly supervised by health personnel. In line with the Essential Drug List for Nepal national guideline (adult= 600 mg base at once, 300 mg base after six hours, 300 mg base on day 1 and 300 mg base on day 2). If other drugs were to be administered, the patient was excluded from the study.

8.4. Criteria for interpretation of test result:

Clinical and parasitological results were classified separately. For overall response, classification of both results were combined and classified in three categories:

- TS= Treatment success
    - Parasite count on day 3 less than day 0 and
    - No parasitaemia on day 7 and day 14 (supported by clinical improvement).
- LTF= Late Treatment Failure
    - Parasite count on day 3 less than day 0 and
    - Increased parasitaemia on day 7 or day 14 as compared to day 3 and/ or
    - Development of signs and symptoms of severe and complicate malaria after day 3.
- ETF= Early Treatment Failure
    - Parasite count on day 3 more than day 0 or
    - Development of signs and symptoms of severe and complicate malaria on day 3 or before.

The above methodological classification slightly different from WHO standard protocol, in the sense that the reappearance of parasites on day 14 was considered to be a LTF and not a reinfection. In other words, the definition adopted in this study was stricter with respect to treatment failure classification.

8.5. Quality control and reliability of the activities:

The principle investigator and a team of physicians were involved seriously with continuous supervision to ensured the quality of the study. Antimalarial drugs used in the test were supplied to the health post by the public health system and it was assumed that quality control

had been performed prior to supply. In order to ensure reliable results, quality control on the reliability of laboratory work and the overall performance of the activities was done.

8.6. Results:

A total of 4039 blood samples were collected from two different areas, 515 (12.7%) were malaria positive in which 130 were confirmed as having *P. falciparum* infection. Out of 130 *falciparum* malaria cases a total of 58 (44.6%) were enrolled (25 patients from Banke and 33 patients from Dhanusha) in the study and followed up. The chloroquine treatment was effective in 51 cases (88%) and was not effective in seven cases (12.%). Among these seven cases Early Treatment Failures (ETF) accounted for two cases (3.4%) and Late Treatment Failures (LTF) accounted for 5 cases (8.6%).

This degree of resistance to chloroquine is still low, which suggests that for the time being the drug should remain the first line drug for the treatment of malaria in Nepal, but it is important to extend testing for chloroquine resistance (using both *in vivo* testing and molecular typing of parasites for accepted chloroquine resistance markers) in different parts of the country.

All treatment failures were successfully treated with sulphadoxine + pyrimethamine (Fansidar^{R}) following the national guidelines of Ministy of Health Nepal.

Result of *in vivo* chloroquine efficacy in *falciprum* malaria infections shown in Table 3.

Table 3. *Falciparum* malaria response to chloroquine.

Age group	TS		ETF		LTF		Total	
	n	(%)	n	(%)	n	(%)	n	(%)
< 10 years	2	(100)	0	(0)	0	(0)	2	(100)
11- 20 years	11	(91.7)	1	(8.3)	0	(0)	12	(100)
> 21 years	38	(86.4)	1	(2.3)	5	(11.5)	44	(100)
Total	51	(88)	2	(3.4)	5	(8.6)	58	(100)

9. *In vitro* cultivation of *Plasmodium falciparum:*

In-vitro cultivation of *P. falciparum* and *in-vitro* assay of anti-malarial drug sensitivity (mainly chloroquine) were evaluated in the different forms. The drug sensitivity assay were evaluated in comparison with ordinary candle jar system and using AnaeroPack Campylo which generate microaerophilic condition. Due to difficulties in long-term maintenance of parasitized blood collected under field conditions during transportation from study site to laboratory, this study had to be discontinued. However, successful cultivation of malaria parasite in sterile glass bottles, and using desiccator containing a candle has been carried out in Tribhuvan University Institute of Medicine, Health Research Laboratory as described previously (Sherchand *et al.*, 1995).

10. Preliminary study on *P. vivax* and chloroquine resistant:

In July and August, 26 patients (8 patients from Bankey and 18 from Dhanusha) infected with *P. vivax* parasites (150 to 1250/µl) found in their blood films were studied. They were treated with 600 mg Chloroquine base by the health post. All these twenty-six patients were requested to visit health post after 3 days, 7 days and 14 days for blood slide examination (both thick and thin smear).

2 patients showed the presence of *P. vivax* (150 to 200/µl) after 3 days; and 1 patients had *P. vivax* (150µl) in day seven and 2 patients showed Parasites (200 and 400 µl) in day 14. These patients had febrile in day 14, since, due to lack of facilities, we could not perform the presence of plasma concentrations of the chloroquine.

Subsequent treatment was given in five patients starting on July 15, 2000 with higher dose of Chloroquine (first day 600 mg, second day 600 mg, third day 300 mg), and Primaquine (7.5 mg three times a day for 14 days). Blood slides were examined on August 26, 2000 and found 2 patients had still blood films positive for *P. vivax* (150 and 400/µl). During the blood examination time one had fever and convulsion. Out of five patients treated with higher dose, 3 patients were cured completely and no appearance of clinical symptoms and their blood slides were also negative. Two patients who had positive malaria were sent to Kathmandu Tribhuvan University Teaching Hospital (TUTH) for admission and treatment.

The resistance of *P. vivax* and *P. falciparum* to chloroquine has increased and spread widely since it was first reported from South-East Asia and South America. In Nepal, there is some documented report on the resistance of *P. falciparum* in Southern Terai, but until now, there had been no documented reports of chloroquine resistance in *P. vivax*. So our preliminary study indicates the presence of chloroquine resistance to *P. vivax* in Nepal. Hence, medical practitioners and other health workers need to be alert to possibility of chloroquine-resistant *vivax* malaria. Further study is necessary to measure level of blood concentration of chloroquine resistant *vivax* malaria and comprehensive study on *P. vivax* resistance to chloroquine in the context of Nepal. Since the predominant malaria in Nepal is *P. vivax*, but improper administration of chloroquine might be a contributory factor to the development of chloroquine-resistant vivax malaria in the district of Nepal. Hence, it is necessary to further investigation. Unfortunately, because of the potentially confounding effects of the host immune response, and because *P. vivax* parasites do not grow in the usual *in vitro* culture system, it has been more difficult to follow the spread of chloroquine-resistant *P. vivax* than chloroquine-resistant *P. falciparum*.

11. A quality control study among malaria health workers/ microscopists.

To measure quality control of microscopy diagnosis on malaria between health post and hospital level, randomly selected batches of stained blood slides with a copy of the workers results were re-examined. The performance of three categories of malaria microscopists was tested.

1. Six recently trained malaria technicians (One weak malaria diagnosis training provided at the beginning of this project).
2. Six government employed malaria technicians from the six-health post.
3. Six government employed malaria technicians from the hospitals.

In addition, individual malaria technicians/ microscopists from different health posts and hospitals were interviewed before collecting malaria slides such as:

1) Working experiences. 2) What kind of method is being used for diagnosis? 3) How many specimens will you process per day?. 4) Have you got recently any training on malaria?. 5) What are the major problems for laboratory diagnosis of malaria?. 6) When you confuse the

microscopic diagnosis of a blood film what do you do?. 7) What is your opinion for the improvement of malaria control programme in Nepal.

11.1. Results:

For the validity of screening microscopist slides, from each batch a standard 2x2 table was prepared, as used by epidemiologist in the analysis of validity of screening tests (Kelsey *et al.*, 1986).

11.1.1. **Six recently trained malaria technicians (One weak malaria diagnosis training provided at the begining of this project).**

A total of 102 slides reexamined of which 23 were malaria positive and 79 were negative. The quality control results did not show any differences between previously reported as positive and negative. The TPR 22.5%; FPR 0%, FNR 0%, 22.5% and accuracy 100%.

11.1.2. **Six government employed malaria technicians from the six health post.**

A total of 60 slides reexamined of which 14 were malaria positive and 46 were negative. The quality control results showed that one previously reported, as negative slides from the health post were found positive with *P. vivax*.

The TPR 30.4%; FPR 0%; FNR 1.7%; EPR 21.6% and accuracy 98.3%.

11.1.3. **Six government employed malaria technicians from the hospitals.**

A total of 75 slides reexamined of which 22 were malaria positive and 53 were negative.

The quality control results showed that 3 previously reported as negative were found positive. Of this 3 positive slides 2 were *P. vivax* and one was *P. falciparum*. The TPR 29.3%; FPR 0%; FNR 13.6%; EPR 29.3% and accuracy 96%.

11.2. Additional findings on the basis of interviews with malaria technicians/ microscopists:

11.2.1. **Working experiences:** 55% of technicians had more than 10 years experience, 30% had less then 5 years experience and 15% had about 2 years experience. Although they had only 2 years experience, they were found fairly competent and capable of malaria diagnosis.

11.2.2. **Methods used for malaria diagnosis:** The majority of malaria technicians/ microscopists (100%) said that they diagnosed malaria by using blood film examination,

however, 15% said that there is a lack of Giemsa stain and microscope slides. Six recently trained malaria technicians mentioned that they use rapid dipstick test when needed.

11.2.3 **Average slides examined:** In the health posts average slides examined were 10 to 20 per day where as in the hospital less than 10 malaria slides examined per day.

11.2.4. **Received training on malaria:** 51.8 % of the technicians received malaria training recently where as 48% said that they had informal training on malaria.

11.2.5. **Problems on laboratory diagnosis of malaria:** Malaria technicians/ microscopists from health posts/ hospitals replied that the supply materials for malaria diagnosis was inadequate, lack of logistic support, unhappy with their remuneration, dissatisfied with their job mainly due to temporary or daily wages. Some of them mentioned that they were unhappy with quality of microscope provided by the government.

11.2.6. **When you confuse the microscopic diagnosis of a blood film what do you do?:** 50% of the health post and 35% of hospitals technicians failed to examine slides when interpretation was difficult. 35% of health post and 50% of hospital technicians said that they requested help to confirm the diagnosis. 15% and 20% of health post and hospital respectively mentioned that they dispatched slides to centre crosschecking office Hetauda Vector borne Disease Research and Training Centre for confirmation, though they hardly ever receive any feedback.

11.2.7. **what is your opinion for the improvement of malaria control programme in Nepal:** Majority of malaria technicians/ microscopists from health post and hospital expressed that they need refresher training on malaria, require adequate salary and security of their job. Some of them revealed need for logistic support and need to be given positive feed back from cross checking centre and district health office.

12. Rapid immunochromatographic OptiMAL assay for detection of *P. vivax* and *P. falciparum* malaria from two malaria districts of Nepal

The study was conducted in two malaria districts (Dhanusha and Kanchanpur) between August 2000 and October 2001 of Nepal to compare the performance of OptiMAL, an immunochromatographic antigen detection assay for the diagnosis of malaria using parasite lactate dehydrogenase (pLDH), against standard *microscopy* in patients with suspected malaria.

The results from the OptiMAL test were compared with those obtained by reading 100 fields of traditional Giemsa-stained thick smear blood films. Whole blood samples as well as thick film of 180 patients suspected of having malaria were received from Dhanusha and Kanchanpur districts. A total of 66 (36.7%) were blood film positive microscopically, while 64 (35.6%) samples were positive with OptiMAL test. The blood films indicated that 80.3% (53 of 66) of the patients were positive for *P. vivax* and 19.7% (13 of 66) were infected with *P. falciparum*. The results demonstrated that the OptiMAL test had sensitivity of 97% and specificity 98%, respectively, when compared with traditional blood films for the detection of malarial infection. Blood samples, not identified by OptiMAL as malaria positive normally contained parasites at concentrations of less than 100 parasites/μl (less than 0.001% parasitemia) of blood. Samples found to have *P. falciparum* were further tested with two other commercially available rapid malaria diagnostic tests, Paracheck (manufactured by Orchid Biomedical system, Goa India) and ICT Malaria P.f. (ICT Diagnostics, Sydney, Australia), both of which detect only *P. falciparum*. Only 10 of the 13 (77%) *P. falciparum*-positive blood samples were identified with ICT and 11 of 13 (84.6%) with Paracheck tests. Thus, OptiMAL accurately identified *P. falciparum* malaria parasites in patient blood samples more often than did the other two commercially available diagnostic tests and established a better correlation with traditional blood films in the identification of both *P. vivax* malaria and *P. falciparum* malaria.

Thus, our study concluded that the OptiMAL malaria test is an useful tool for the rapid diagnosis of malaria and an easy alternative to existing tests.

13. Malaria infection: a preliminary study among Terai people who crossed or never crossed Southern border of Nepal

The current study was conducted in Kanchanpur districts of Nepal among adults suspected malaria patients with or without traveled (cross-border) history.

All suspected cases of malaria from district hospital of malaria clinic in the period of August and December, 2001 were investigated among two population group, who were identified on the basis of travel history: group 1 included person who frequently visited India or crossed Southern border and group 2 included person who never traveled to India or never crossed Southern border but visited within the country.

A total of 438 adult patients (age16 or above) were included in which 256 people who crossed the border and 182 people who never crossed the border.

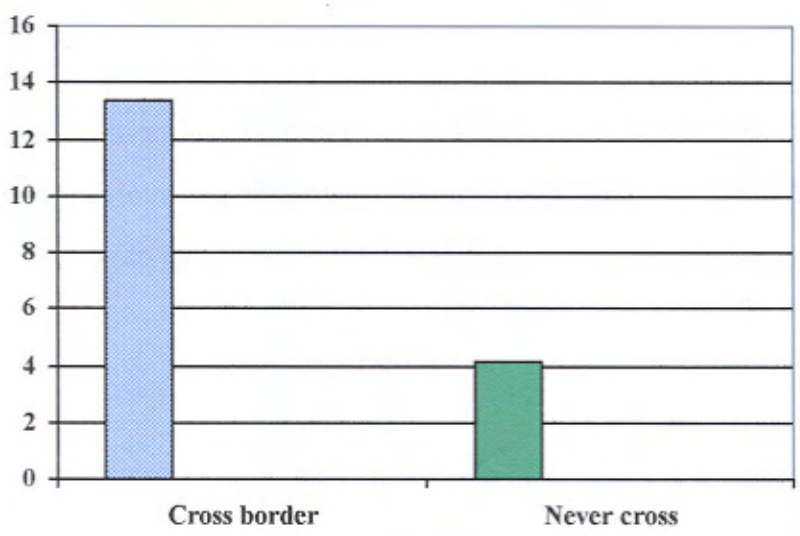
Malaria diagnosis was made based on examination of Giemsa stained thick and thin blood smears. The study populations were asked their travel history, clinical history, Chloroquine intake, along with knowledge, attitude and health seeking behaviour to ward malaria infection.

13.1. Clinical examination and blood collection: All patients were clinically examined by a physician and made provisional diagnosis. Finger prick blood as taken from each patient and two pairs of slides (thick and thin) was made for microscopy examination. All stained slides were examined independently by two qualified Medical Laboratory Technicians of Malaria clinic of Kanchanpur District Health Office and Tribhuvan University Teaching Hospital, then further confirmed by the investigator himself.

13.2. Results:

A total of 438 suspected malaria were examined during the study period of whom 198 (45.2%) were female and 240 (54.8) were male. The age ranged from 16 to 68 years in which 256 people who frequently visited India or crossed the southern boarder. The proportion of malaria infection depicted in Figure 5.

Figure 5. Proportion of malaria cases between two groups.



13.3. Prevalence of parasitaemia:

The prevalence of parasitaemia was 9.6% (42/ 438) in which 18 cases were *P. falciparum* and 24 cases were *P. vivax*. Of 256 patients who crossed the border 34 (13.3%) had parasitaemia in which 44.1% (15/34) were *P. falciparum* infection. The proportion of falciparum malaria infection among the patient who frequently crossed the southern border (83.3%)15/18 and who never crossed the border 16.7% (3/18) was significantly difference ($P < 0.05$). Although numbers are too small to draw any significant conclusions the study suggest a greater risk of infection in migrants, a feature which may play a role in malaria epidemiology and the spread of drug resistance to Nepal.

13.4. Result from the interview:

Symptoms:

Of the patients who reported fever, headache, chills, vomiting, joint pain, diarrhoea 14.5% (18/124) patients were found malaria positive compared to the patient who only complain fever and headache 5% (8/145). The prevalence of parasitaemia among patients complaining of intermittent fever, respiratory infection with breathing difficulties and chest pain 7.9% (11/139) had malaria parasites in their blood. Patients with fever and liver diseases-jaundice/hepatitis carried parasites in 11.1% (2/18) while patients without liver disease but fever, unconscious and suspected encephalitis were positive for malaria parasites in 25% (3/12). All parasitaemia cases in this group of patients (with liver disease and encephalitis) showed *P. falciparum* infection who had frequently crossed the border of Southern Nepal.

The validity of the patients self appraisal of their illness when asked "Do you think you have malaria?" showed that patients who answered 'yes' had a higher risk of being parasitaemia 12.9% (24/186) than patients who replied 'no' or 'I don't know' 7.1% (18/252), but the difference was not significant.

Information on the patient's personal medical history such as past history of illness, chronic health problems, previous examination in the clinic and frequent health examination did not show an association with parasitaemia (all $P > 0.05$).

13.5. Age and sex wise distribution of parasitaemia:

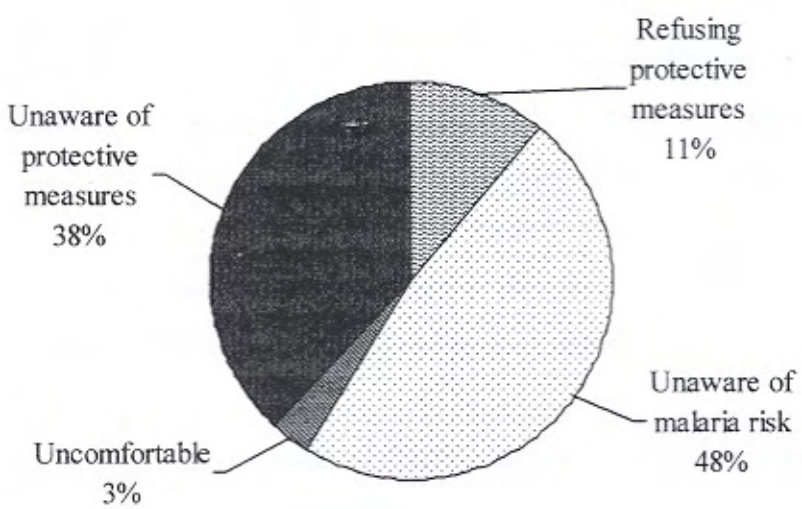
Comparison of different age and sex groups with regard to malaria infection showed no association with parasitaemia. However, the age between 16 to 20 years of male patients had high rate 14.3% (6/42) of parasitic infection.

13.6. Attitude and practices towards malaria prophylaxis:

The analysis of the cases of malaria patients who frequently crossed the Southern border in this study 80.9% (34/42) showed high rate of malaria infection mainly *P. falciparum* with low intake of anti-malaria chemoprophylaxis. On the basis of prospective questionnaire survey among 256 people who frequently visited India or crossed the Southern border. The length of stay during visit was 3 days to 6 months. 183 patients (71.5%) reported correct knowledge of malaria risk. A small number of patients had taken pre-travel chemo prophylaxis (9%) while 91% of the people did not take pre-travel advice and chemo prophylaxis. 25% people used some protective measures such as bed nets, mosquito repellent, mosquito coils and screening of windows and doors whereas 75% did not. The reason for not taking pre-travel chemoprophylaxis and preventive measures is shown in Figure 6. These included unawareness of malaria risk (48%), unaware of protective measures (38%), refusing protective measures (11%) and due to uncomfortable (3%). Hence, education and strong motivation is needed to adopt such preventive measures.

All people who cross the border to malarias areas need to be aware of the risk of infection, how best they can protect themselves and where they can seek urgent medical advice if they get a fever.

Figure 6: Reasons for not taking protective measures



14. Constraints and recommendations for the research needed:

14.1. Constraints:

According to the national antimalaria programme proposed guidelines epidemiological data, meteorological data, natural calamities, development activities, movement of population have to be recorded and analysed for delineation of affected area and identification of high risk group to forecast epidemic, early preparedness and timely intervention measures to prevent both mortality and morbidity associated with outbreak of the disease. However, poor or inadequate surveillance, information management system and lack of laboratory services and inadequate capacity building at district level results in a backlog of slides, accumulation of unanalyzed data, and, subsequently, vulnerability and high risk groups are not identified timely. In addition to this many staff posts at different levels remain vacant for several years and it is practically impossible to cover the target population. Moreover, in addition to inadequate motivation and inadequate inter-sectoral coordination, and the current state of insurgency and movement of population create such difficulties that health personnel are scared to visit the insurgency areas resulting in failure of intervention measures, which risk to lead to an unprecedented increase of malaria incidence.

Currently, the activities of the National Malaria Control Programme are coordinated by the Vector Borne Disease Research and Training Centre (VBDRTC) in Hetauda, with support from the Environmental Health Project (EHP), a USAID-funded INGO. The activities of this international NGO are concentrated on malaria, JE and kala azar. VBDRTC and EHP are working on routine active case surveillance and sociomedical studies in selected districts, as well as patchy in vivo drug resistance studies in malaria. They had initially planned to strengthen the national capability in laboratory services and the development of human resources in the country. However, due to the lack of available expertise and financial constraints, this objective has not been achieved so far. Currently, our laboratory (Tribhuvan University Institute of Medicine, Health Research Laboratory) is the only one in Nepal to have facilities for the cultivation of *P. falciparum in vitro* and where molecular studies can be performed. It is indicated that our laboratory, in collaboration with the National Malaria Control Programme, would provide the necessary infrastructure and technical expertise for the research needs of the programme, in particular for the molecular surveillance of the spread of drug resistance in the country.

14.2. Research needed: on the basis of existing situation of the country:

The epidemic cycle consists of pre-epidemic, epidemic, post-epidemic and inter-epidemic waves. Of them, pre-epidemic phase (incubation period) is most important because during this phase

epidemiological foci multiply and show gradual upward trend of malaria incidence. It may be due to either high density of vector species or increased ratio of gametocyte carriers. The combination of both events is responsible for the creation of epidemic foci in a given ecosystem. The impact of various epidemiological indicators may vary under different micro- and macro-ecosystems. Therefore, it is suggested that longitudinal studies should be carried out in different epidemiological paradigms to elucidate the functional relationship between different epidemiological determinants for the accurate prediction of epidemics.

In view of the advancements in computer and communication technology, geographical information system (GIS) should be explored to identify epidemic prone areas with the help of good quality topographical, political and thematic maps.

In Nepal, entomological components are quite inadequate or non-existent at primary health centers. There is also no infrastructure for surveillance of vector borne diseases particularly in inaccessible areas. Operational studies are required to identify minimum epidemiological, social and environmental indicators using sampling methods to estimate true incidence of malaria for accurate and timely prediction particularly in high risk areas. There is also an urgent need to develop mathematical model to monitor inoculation rate, disease burden, immunity status, disability, adjusted life years and impact of intervention measures in epidemic prone areas.

Early detection and prompt treatment (EDPT) is one of the most important components of the revised global strategy for malaria control. Therefore, increased emphasis should be placed on rapid diagnostic techniques, which are highly sensitive, species-specific and cost-effective and may be produced with indigenous technology. Studies on seroepidemiological techniques should also be undertaken to measure immune-status and to assess the impact of intervention measures particularly during ascending trend of epidemic.

Studies are also required to monitor drug and insecticide resistance at district level to detect failures of intervention measures, selection of appropriate strategic measures particularly on onset of pre-epidemic period to prevent the epidemics in stable malarious areas.

Population movement for economic reasons is a serious problem of Nepal. The continuous influx of population from endemic areas or vice-versa create epidemic foci and also responsible for spread of drug-resistant strains of falciparum malaria. Studies should be carried out to study the migration pattern and containment of drug-resistant malaria strains. Molecular studies must be undertaken to study the genetic diversity of drug susceptibility using the whole range of available markers (include Pfcrt, Pfmdr, PfDHFR and PfDHFS), particularly at a time when chloroquine resistance appears to be on the increase and when SP starts to be used.

Because of ecological, genetic and social diversities, the techniques developed in particular ecosystem may not be applicable at national, regional and global level as malaria is a local and focal disease. Therefore, meta-analysis of studies is pre-requisite before recommending or adopting any technique in a global programme

14.3. Research needed: on the basis of current study under TDR/ RCS grant:

The aim of this study was to contribute to the understanding of why cerebral malaria does not appear to be a substantial cause of malaria mortality in Nepal, despite a low level of endemicity, normally insufficient to induce an effective premunition in the population. The hospital admission study clearly established that malaria cases were grossly underdiagnosed. The finding that neurological complications of malaria are frequently not recognised as symptoms of malaria is a suggestive indication of a more serious problem: the misdiagnosis of cerebral malaria. In a country where *P. vivax* used to represent 90% of malaria cases in the early 1970s and is still the dominant species today, the equation of the Vivax paroxysm symtomatology and malaria is not surprising. The finding, in literature on Japanese Encephalitis published by the Ministry of Health of Nepal (Bista et al., 1999), of the description of an algorithm for febrile coma, which leads to a diagnosis of either Japanese Encephalitis (JE), or meningitis, without mentioning cerebral malaria as an option, reflects a deeply engrained misconception amongst health specialists in the country. The data collected from 5,220 admissions over a period of 16 months are suggestive: of the 142 cases of *P. falciparum* observed at hospital level, 27 were clustered in the "encephalitis/CNS manifestations" group. Tragically, once the patient is referred to a hospital with this misdiagnosis, the diagnosis will only rarely be reversed because, even when laboratory facilities are available, the microscopists in the hospital will not read malaria slides (since this is a prerogative of the "malaria microscopist" at the district health post) and the patient will not receive anti-malarial treatment. A percentage of these patients will die without this mortality appearing in malaria statistics for Nepal since most will be wrongly reported as JE in official records. The possible confusion of cerebral malaria and JE in the Terai region of Nepal, an area where both diseases are clearly endemic, has no obvious solution. In a study in the Mid-Western region of Nepal, we recently followed 187 patients referred to the district hospital with the diagnosis of JE and found that 23 (12.3%) of these had a positive malaria slide with a relative *P. falciparum* frequency of 74%; in contrast, only 119 (64%) had a positive serology for JE and, even of these, 15/119 actually were malaria slide positive (Sherchand *et al.*, 1998). Differential diagnosis is not possible on clinical grounds alone (at least not with the resources available at district hospital level), there

is no easy laboratory test for JE available at the peripheral level and even the finding of malaria positive slides does not necessarily exclude concomitant JE. However, since JE is not treatable and leads to a case fatality of up to 60%, it would be reasonable to recommend emergency microscopy in all cases of febrile coma to exclude Falciparum malaria before accepting the JE diagnosis. Unlike JE, cerebral malaria is a treatable condition, as long as it is recognized in time and as long as injectable antimalarials are readily available. Since cerebral malaria does not officially exist or is, at best, considered to be an exceptional occurrence, hospitals and private pharmacies in Nepal rarely stock quinine or artemisinin, which compounds the problem.
The most difficult test will be to convince the trainers of medical officers to abandon the inappropriate diagnostic algorithm for febrile coma and this can only be achieved by firm policy statements.

Another difficult question is to convince policy makers to make better use of the expertise of malaria microscopists. Our studies have confirmed that there is here an excellent, well-trained body of competent technicians, whose time is essentially wasted in reading "*a posteriori* slides" (i.e. where the result of reading the slide comes too late to serve a purpose for the management of malaria cases) rather than to be fully integrated in the health service. The move of the microscopists to the district hospital (which we had suggested in our original proposal) may not be the adequate solution in all situations but would need to be further investigated. In any case, it will be necessary to make sure that medical officers and technicians clearly understand that the reading of slides needs to be done immediately if this is to be of benefit to *P. falciparum*-infected patients. It will also be necessary to emphasize this need for urgent microscopy to laboratory technicians in reference/district hospital and make them understand that reading malaria slides cannot be the prerogative of malaria microscopists, as is currently the case in many districts. Some re-training of hospital laboratory technicians may be needed (something malaria microscopists could be involved in).

As a component of this project, it was necessary to examine whether some of the new antigen detection assays may be used as an alternative to microscopy for case management. This may be a possible alternative to microscopy in emergency situations and should be systematically used to detect *P. falciparum* malaria in all suspected epidemics of "encephalitis" in the Terai district.

A study of symptomless vivax malaria infections indicated that a fraction of the native population may be resistant to malaria and may serve as reservoirs of the infection. Precise detection and diagnosis of Plasmodium species in blood is of major importance in determining the

treatment of the individual as well as control approaches for the community. PCR-amplification of ribosomal DNA is a very sensitive and species-specific diagnostic method for malaria that detects only living parasites. The expertise acquired in this study may now be applied to other research questions where PCR is the best experimental approach.

The preliminary study indicated a resistance of *P. vivax* and *P. falciparum* to chloroquine. Though the degree of resistance to chloroquine is low, it is important to expand the detection of chloroquine resistance (using both *in vivo* testing and molecular typing of parasites for accepted chloroquine resistance markers) in different parts of the country.

15. Publications on Malaria:

During my study under WHO-TDR fellow-ship and TDR-RCS grant, I have presented and published following research papers:

15.1. Sherchand JB, Hommel M, Shrestha MP. Study of Malaria in Southern Nepal: Use of ParaSight-F test. Presented in 8th Malaria Meeting of *British Society for Parasitology*, University of Glasgow UK.1996; 39.

15.2. Sherchand JB, Shrestha MP, Hommel M, Ohara H, Shrestha BL, Sherchand S. Resurgence of Malaria in Southern Nepal: A Study of Socio-Medical Aspects. *Environmental Sciences, An International Journal on Environmental Physiology and Toxicology.* 1996; **4:** 55-70.

15.3. Sherchand JB, Shrestha MP, Ohara H. Measurement of malaria endemicity in relation to socio-medical aspects of malaria control in Nepal. Paper presented in *XIV International Congress for Tropical Medicine and Malaria.* "New Goal for the 21st Century" Nagasaki, Japan Nov. 17-22, 1996; 332.

15.4. Sherchand JB, Shrestha MP, Shrestha BL, Banerjee MK, Shakya S. A preliminary study on field trials with insecticide-treated mosquito-nets for malaria control in rural endemic communities of Nepal. *J Nep Med Assoc;* 1995; **33:** 195-203.

15.5. Sherchand JB, Hommel M, Shrestha MP. Use of *in vitro* culture *Plasmodium falciparum* antigen for measuring antibodies to malaria. *J Inst of Med* 1995; **17:** 78-85.

15.6. Sherchand JB. Detection of malaria endemicity in rural communities of Southern Nepal: A seroepidemiological survey in 2-9 year old children. Paper presented and published in Souvenir of Second congress of Association of clinical Pathologists of Nepal. Kathmandu Nepal., Nov. 7-8, 1997; **2:** 44-47.

15.7. Sherchand JB, Shrestha MP, Hommel M, Ohara H. Hospital based study in Southern Nepal on morbidity of malaria. *Khoj-Bin: Journal of Nepal Health Research Council* 1998; **2:** 18-22.

15.8. Sherchand JB, Shrestha MP, Jimba M. Malaria and Health Information System in Nepal. *Journal Nepal Public Health Association* 1998; **1:** 17-19.

15.9. Sherchand JB, Jimba M, Shrestha MP. Occurrence of Japanese encephalitis virus in mid-western region of Nepal: A sero-epidemiological studies on clinically diagnosed encephalitis in relation to severe malaria. *Journal of the Nepal Association for Medical Laboratory Sciences* 1998; **1:** 39-44.

15.10. Sherchand JB, Shrestha MP, Ohara H. Identification of malaria endemicity in rural areas of Nepal: A malariometric and seroepidemiological study in children. *Journal of the Inst. of Med.* 1998; **20:** 164-175.

15.11. Sherchand JB. Serological Diversity to Blood stage Malarial antigens from Endemicity of rural Nepal in relation to Identification of protective antibodies. *J. Nepal Biotechnology Association* 1999; **2:** 1-4.

15.12. Sherchand JB and Hommel M. Can *ParaSight-F* be used for rapid diagnosis of Falciparum malaria in Nepal ?. *Journal of the Inst of Med* 1999; **21:** 201-206.

15.13. Bajracharya P., Sherchand JB & Sharma A. Serodiagnosis of *Japanese encephalitis* and *malaria*: An assessment of public health awareness about the above diseases (a study confined within Bheri Zonal Hospital. (Submitted- *Journal Inst. of Med.* 2001).

15.14. Sherchand JB. OptiMal assay for detection of malaria infection in Nepal. *Acta Tropica* 2002; 83: 59.

15.15. Sherchand JB. Hommel M. Inadequate algorithms for febrile coma misdiagnose cerebral malaria in Nepal (manuscript submitted in International Journal, 2002).

16. Major development during the reporting year:

16.1. Material Resources:

After obtaining TDR research grant, we developed our Department of Microbiology & Health Research laboratory; expanded physical space, improved equipment and developed necessary manpower. Two students of Master degree in Microbiology were supported by this project to carry out their thesis on (i) Study on *Japanese encephalitis* related to severe malaria and bacterial meningitis in mid-western Nepal and (ii) Study on *Plasmodium vivax* and *P. falciparum* resistance to chloroquine from malaria endemicity of Nepal.

16.2. Staff development: We increased the number of trained manpower in our institution laboratory to work on (i) diagnosis of malaria, (ii) immunological assay and PCR technology. In addition, we trained and guided to other faculty members on malaria research and also established linkage with national and international researchers in the field of malaria and tropical diseases.

17. Visiting experts

17.1. **International:**

17.1.1. Professor Marcel Hommel, Liverpool School of Tropical Medicine, UK.

17.2. National experts:

17.2.1. Professor Kin Yon Sohn, Department of Biochemistry and Molecular Medicine Research Laboratory, Tribuvan University, Institute of Medicine Kathmandu Nepal.

17.2.2. Associate Professor N.R. Tuladhar, Head, Department of Microbiology Tribhuvan University Institute of Medicine, Maharajgunj, Kathmandu Nepal.

18. Linkage established:

18.1. The University of Liverpool, School of Tropical Medicine, Division of Molecular biology and Immunology, **Professor Marcel Hommel**

18.2. Nagasaki University, School of Tropical Medicine, Department of Protozoology, Nagasaki Japan, **Professor H. Kanbara.**

18.3. Kyorine University School of Medicine, Department of Infectious disease, Tokyo, Japan, Dr. **Kosuke Haruki**, Senior Lecturer.

18.4. Uniformed Services University of the Health Sciences, Bethesda, Maryland, U.S.A., **Professor John H. Cross.**

19. Additional funding:

Additional fund not available, however some support to conduct the study HMG/JICA/JMA School Health Project in some district of Nepal provided vehicle and antimalarial drugs.

Though this project was no longer funded in 2001, we carried some work in two districts and included the findings in the report.

20. *Acknowledgements:*

This study was supported by UNDP/ World Bank/ WHO Special Programme for Research and Training in Tropical Diseases (TDR-RCS Grant).

21. References:

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Annexes:

Annexe 1: Description of study districts (Dhanusha, Banke and Kanchanpur)

1.1. Dhanusha district: The district is bounded on the west by Mahottari district, on the east by Siraha district, on the north by Sindhuli district, and on the south by Bihar (India). This district covers an area of 221,746 hectares, and is divided into one municipality and 101 village Development Committees (VDC). The District Headquarters is at Janakapurdham.

This is a Terai district consisting largely of flat alluvial plain. The elevation ranges from 61 to 610 meters. The district has two types of climate: tropical and sub-tropical. The temperature varies from an average minimum of 19.3^0 C in the winter to average maximum of 30.5^0 C in summer. The average rainfall is 1479 mm.

Total population of the district is 543672 of which 51.8% male and 48.2% female. The majority of population is Yadav/ Ahir (20.9%) followed by Muslim (7.6%), Kewata (6.1%), Brahmin (5.6%), Sudhi/ Kalwar (5.5%), Teli (5.1%), Dhanuk (4.7%), Kesahawa (4.6%), Chamar (3.5%), Mallah (2.9%) and other (33.4%). The district has three hospitals with 13 health posts. Major occupation 72.2% engaged in agriculture. Less than 18 % of the people are production laborers, sales workers, professional/ technical workers, clerical and service workers.

1.2. Banke district: The district is bounded on the west by Bardia district, on the east by Dang district on the north by Salyan and Surkhet districts, and on the south by UP (India). The district covers an area of 235,982 hectares, and is divided into one municipality and 46 VDCs. The district Headquarters is at Nepalganj municipality.

This is a Terai district consisting largely of flat alluvial plain. The elevation ranges from 129 to 1,290 meters. The district has two types of climate: tropical and sub-tropical. The temperature varies from an average minimum of 16.3^0 C in the winter to average maximum of 30.9^0 C in summer. The average annual rainfall is 1263.6 mm.

Of the total land area 25% is agricultural land, 21.8% is under cultivation, 3.2% is non cultivated land and 1.1% is grassland, 70.9% is forest and remaining 3.0% is covered by sand, gravel, boulder, and water bodies. Only 20.2% of the total irrigable land was under irrigation. The total population of the district is 285,604 of which 51.7% are male and 48.3% female. The majority of the population is Muslim (20.0%) followed by Tharu (16.0%), Chhetri (10.9%), Brahmin (5.9%), Magar (5.4%), Yadav/ Ahir (4.7%), Kami (4.7%), Kurmi (3.2%), Thakuri (2.3%), Chamar (2.1%) and other (28.8%).

The district has three hospitals and 11 health posts. Major occupation is agriculture (68%) and less than 33% of the people are production laborers, sales workers, professional/ technical workers, Clerical and service workers.

1.3. Kanchanpur district: The district is bounded on the west by (UP) Inida, on the east by Kailali district on the north by Dadeldhura districts, and on the south by UP (India). The district covers an area of 163678 hectares, and is divided into one municipality and 19 VDCs. The district Headquarters is at Mahendranagar municipality.

This is a Terai district consisting largely of flat alluvial plain. The elevation ranges from 176 to 1,528 meters. The district has two types of climate: tropical and sub-tropical. The temperature varies from an average minimum of 17.2^0 C in the winter to average maximum of 31.5° C in summer. The average annual rainfall is 1263.6 mm.

Of the total land area 25% is agricultural land, 21.8% is under cultivation, 3.2% is non cultivated land and 1.1% is grassland, 70.9% is forest and remaining 3.0% is covered by sand, gravel, boulder, and water bodies. Only 20.2% of the total irrigable land was under irrigation. The total population of the district is 382290 of which 51% are male and 49% female. The majority of the population is Muslim (20.0%) followed by Tharu (16.0%), Chhetri (10.9%), Brahmin (5.9%), Magar (5.4%), Yadav/ Ahir (4.7%), Kami (4.7%), Kurmi (3.2%), Thakuri (2.3%), Chamar (2.1%) and other (28.8%).

The district has one hospitals, 2 Primary Health Care Centre, 8 health posts and 11 Sub-health posts. Major occupation is agriculture (65%) and less than 35% of the people are production laborers, sales workers, professional/ technical workers, Clerical and service workers.

Annexe 2: Clinical history of hospital patients and malaria cases (Dhanusha)

Clinical diagnosis: (on the basis of patient's history)	**Prevalence of malaria parasites**				
	No.	***P. vivax***	***P. falcip***	***P. malariae***	**Total MP+(%)**
Fever and Headache	147	103	17	2	122 (83)
Chill/ rigor(malaria suspected)	87	27	8	0	35 (40.2)
Vomiting	71	5	3	0	8 (11.3)
Convulsions	35	18	12	0	30 (85.7)
Gastro-enteritis/ diarrhoea	273	1	1	0	2 (0.7)
Hepatitis/ Jaundice	62	14	7	0	21 (33.9)
Typhoid	186	16	7	1	24 (13.0)
Respiratory infection	194	21	3	0	24 (12.3)
Encephalitis/CNS manifestation	71	13	11	1	25 (35.2)
Meningitis	81	12	3	0	15 (18.5)
Measles	31	0	2	0	2 (6.4)
Septicaemia	31	0	0	0	0 (0.0)
T.B.	74	6	1	0	7 (9.4)
Genito/ urinary infection	105	0	0	0	0 (0.0)
Pregnancy/ Ectopic pregnancy	8	1	2	0	3 (37.5)
Kalaazar/ suspected	33	4	2	0	6 (18.2)
Malnutrition	51	8	4	2	14 (27.4)
Suspected cases of cancer	9	0	0	0	0 (0.0)
Eye infection	51	0	0	0	0 (0.0)
Skin infection	114	8	4	0	12 (10.5)
Others *	107	1	0	0	1 (0.9)
Unknown	37	3	0	0	3 (8.1)
Total cases	1858	261 (14.0%)	87 (4.6%)	6 (0.3%)	354 (19.1%)

* Others: Diabetes mellitus, hypertension, surgical cases, tetanus and snake bite.

Annexe 3: Clinical history of hospital patients and malaria cases (Banke district)

Clinical diagnosis: (on the basis of patient's history)	**Prevalence of malaria parasites**				
	No.	***P. vivax***	***P. falcip***	***P. malariae***	**Total MP+(%)**
Fever and Headache	154	32	9	0	41 (26.6)
Chill/ rigor(malaria suspected)	93	13	3	0	16 (17.2)
Vomiting	69	2	1	0	3 (4.3)
Convulsions	48	8	5	0	13 (27.1)
Gastro-enteritis/ diarrhoea	272	3	0	0	3 (1.1)
Hepatitis/ Jaundice	66	5	1	0	6 (9.1)
Typhoid	171	3	2	1	6 (3.5)
Respiratory infection	214	9	2	0	11 (4.6)
Encephalitis/ CNS manifestation	159	2	13	0	15 (9.4)
Meningitis	89	6	1	1	8 (9.0)
Measles	33	0	1	0	1 (3.0)
Septicaemia	17	0	0	0	0 (0.0)
T.B.	68	2	1	0	3 (4.4)
Genito/ urinary infection	113	2	0	0	2 (1.7)
Pregnancy/Ectopic pregnancy	11	1	1	0	2 (18.2)
Kalaazar/ suspected	17	1	0	0	1 (5.8)
Malnutrition	60	5	3	0	8 (13.3)
Suspected cases of cancer	13	0	0	0	0 (0.0)
Eye infection	83	0	0	0	0 (0.0)
Skin infection	187	11	0	0	11 (5.8)
Others *	193	10	0	0	10 (5.1)
Unknown/ unrecorded	51	1	0	0	1 (1.9)
Total cases	2181	116 (5.3%)	43 (1.9%)	2 (0.09%)	161 (7.4%)

* Others: Diabetes mellitus, hypertension, surgical cases, tetanus and snake bite.

Nepal Health Research Council

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Date:

Ref: 250

October 21, 1997

Dr. Jeevan Bahadur Sherchand
TU, Institute of Medicine
PO Box :10404
Kathmandu

Subject : Approval of the research proposal entitled as **The value of malaria diagnosis : A Preliminary Study in the Terai District of Nepal**

Dear Dr. Sherchand:

We are pleased to inform you that the above said research proposal has been approved by the NHRC board on the date of October 1,1997 (Asoj 15, 2054).

Congratulations

Thank you

Your sincerely

Dr. Ram Prasad Upreti
Member-Secretary

Phone # (01) ~~216895~~ 254220 Email:NHRC@npl.healthnet.or g Fax # 977-1-228069 Ram Shah Path, P.O.Box 7626, Kathmandu, Nepal

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. Ram Kewal Shah

presentative
Ministry of Finance
tional Planning Commission
Ministry of Health
ief, Research Committee, IOM
airman, Nepal Medical Council

April 10, 2000.

Dr. Jeevan B.Serchand
Associate professor
Department of Medical Microbiology and Parasitology
Maharajgunj

Subject: Approval of the research proposal entitled **"The value of malaria diagnosis: A Preliminary Study in Terai District of Nepal ."**

Dear Dr. Serchand:

We are pleased to inform you that the changes made to the above mentioned proposal submitted by you has been approved by NHRC board on the date October 1, 1997, following the recommendation of the Technical Review Committee (TRC) and Ethical Review Committee (ERC). This also certifies that the proposal is ethically cleared.

As per NHRC regulation you are to follow strictly the protocol stipulated in your proposal finalized after the interaction of TRC and ERC with you. Any change in objectives (s), problem statement, research question or hypothesis, methodology, implementation procedure, data management and budget that may be necessary in course of the implementation of the research proposal can only be made so and implemented after prior approval from this council. You are thus strongly advised to submit to NHRC the details of such changes intended or desired with justification prior to instituting actual change.

You are also to abide by the ethical guidelines of NHRC strictly during the implementation of your research proposal. In addition, in course of investigation of any medical health problem needing immediate care, further investigation, or expert consultation you are obliged to inform the subject-study or control clearly in writing from within seven days of detection. However, during the transmission of such information confidentially must be maintained.

Lastly, you are obliged to submit periodic progress reports every 3 months and submit three copies of the final research report, and financial statement after completion of the research. If an article based upon that research is published, you should submit two copies of that article.
If you have any question, please contact our research officers.

Thank you.

Yours truly,

Dr. Kamal Gyawali
Member-Secretary

Tel. (977-1) 254220, Fax: 977-1-262369, Email: NHRC@healthnet.org. np Ramshah Path, P. O. Box 7626, Kathmandu, Nepal.

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1976 Certificate in Medical Science (Health lab)
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1980 BSc MT, Postgraduate Institute of Medical Education & Research Chandigarh, India
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1991 MSc Trop. Med. Mahidol University, Faculty of Tropical Medicine, Bangkok, Thailand
1996 Ph.D. Trop. Med. The University of Liverpool, School of Tropical Medicine, UK.
1998 Postdoct. Parasitic immunology, Kyorine University School of Medicine, Tokyo Japan

Present and Past Positions:

1976-1984 **Instructor:** Tribhuvan University Institute of Medicine
Department of Pathology and Health laboratory Kathmandu Nepal.
1984-1993 **Asst. Lecturer:** Tribhuvan University Institute of Medicine, Department of Community Medicine, Public Health Research Laboratory.
1993-1999 **Lecturer:** Tribhuvan University Institute of Medicine. Dept of Community Medicine.
2000 on ward **Associate Professor:** Tribhuvan University Teaching Hospital, Department of Microbiology-Parasitology and Health Research Laboratory, Nepal

Association with Professional and advocacy organisation:

1997-date **Executive Director:** Infectious and Tropical Diseases Research and Prevention Centre, Nepal
1996-1998 **Consultant Epidemiologist:** Nepal Health Research Council
1997-1999 **Consultant Infectious Diseases Control:** HMG/JICA/JMA Community and School Health Project Kathmandu Nepal
1997-2000 **Consultant:** National Micronutrient survey/ Baseline survey for World Food Programme in Nepal, HMG/UNICEF/WFP/NewERA-Nepal
1997 onward **Advisor:** Basic Skill Human Resources Development Centre/ Nepal
1998 onward **Vice President:** Nepal Bio-technology Association
2001 onward **Executive Board Member Biotechnology:** Ministry of Science and Technology

Awards and Honours:

2002 **Dirgh Sewa Padak Medal:** From Vice Chancellor of Tribhuvan University.
1989 **Chitra Pahari Scientific Medical Award:** For the Best Research work.
1998 **Mahendra Bidhya Bhusan Class-A Medal**: by His Majesty King of Nepal.
1996 onward **Honorary Research Fellow:** Liverpool School of Tropical Medicine, UK
1977-80, 93-96 **UNDP/World Bank/ WHO-TDR Fellowship**: for BScMT & PhD degree.
1997 onward **Parasitic Research Collaborator:** Uniformed Services University of the Health Sciences, Bethesda, Maryland USA.
1986, 1998 **JICA and AIE Fellowship**: for Research study on Infectious Diseases, Japan

Publications:

- More than 80 scientific research papers published in international and national journals
- Published academic books and many reports on research and evaluation.