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Precision mosquito survey using GIS : A case study of *An. minimus* – A foothill vector of malaria in India

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In India anopheline fauna comprises of 58 morphologically distinguishable species. Of these, six are major vector and three are secondary vector species of malaria. Major vector species consists of *Anopheles culicifacies, An. fluviatilis, An. stephensi, An. dirus*, *An. minimus* and *An. sundaicus*. It has been observed that the mosquito species establish their population in different ecological settings which are most suited for their survival and longevity[1]. The increasing trend of rapid changes in environment leading to changing vector distribution demands frequent surveys to monitor change in vector distribution. Revised malaria control strategy also laid emphasis on selective integrated control methods. These are situation specific and need a detailed knowledge of local vectors[2]. Remote sensing (RS) and geographic information system (GIS) have opened a new horizon for monitoring and control of diseases. Remote sensing helps in generating new information and update the old ones and GIS, a computer assisted system facilitate inputting, processing, analyzing, integrating and presentation of both spatial and non-spatial information.

Wood *et al*, at NASA, using GIS capabilities identified high and low anopheline producing rice fields in California two months before peak larval season with nearly 90% accuracy[3]. In Israel, distance between vector breeding sites and migratory population centers were correlated with malaria incidence using GIS[4]. Flooded pastures and transitional swamps were identified as important land elements for potential vector breeding sites using GIS[5]. Malaria information system (MIS) had been also used to identify malarious areas and risk factors in KwaZulu/ Natal province in South Africa[6]. Scientists have shown significant association between age related malaria infection in Gambian children and environmental greenness[7]. GIS has been also used to plan malaria control programme in South Africa[8]. In Africa the number of people at risk of contracting malaria as well as the yearly interventions cost were estimated using GIS methods and the disease epidemiologic characteristics[9]. The spatial distribution of malaria cases in Anhui province of China showed correlated increased risk for

malaria with the spatial cluster sizes[10]. A GIS based prediction model for malaria was developed in Nigeria to enhance the cost effective control efforts[11]. Similarly lower intervention coverage, and lower adherence were identified in villages in Khammouane province, Laos and appropriate control strategies were suggested[12].

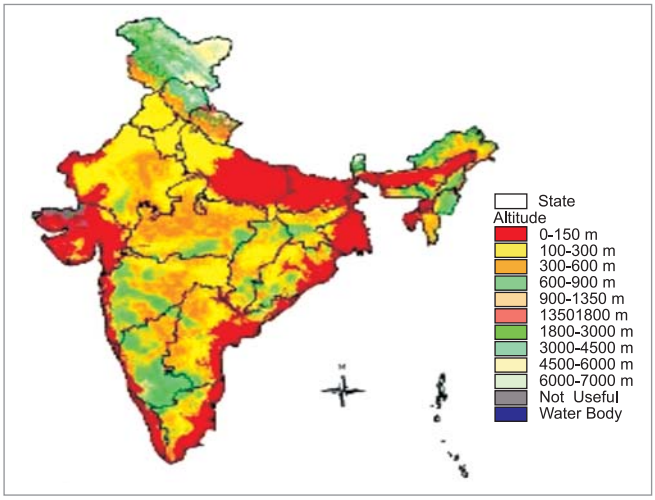
The RS and GIS have been used in India also to estimate mosquito production, mapping of malaria receptive areas and identify hot spots and malaria risk factors in different areas[13-16]. Further, favorable areas for distribution of Indian anophelines including six major malaria vectors have been also mapped using GIS[17-19]. In the present write-up an attempt has been made to present a case study of *An. minimus*, a vector of malaria of foothill area with respect to GIS, its usefulness in precision mosquito surveys and decision support in malaria control.

Distribution of *An. minimus*

An. minimus is the most important vector of malaria along the foothills of Himalayas from Uttarakhand to northeastern part of India. The species breeds in streams, ditches, channels, tea garden drains, *etc*. and prefers to breed in clear unpolluted slow-moving water with grassy and particularly shaded edges. This is essentially a species of hill and foot-hill areas in tropical monsoon regions. Because of its occurrence in forested areas in foothills, out of several ecological parameters that govern its distribution five *viz* forest-cover, soil type, altitude, temperature and rainfall have been considered important and are described here.

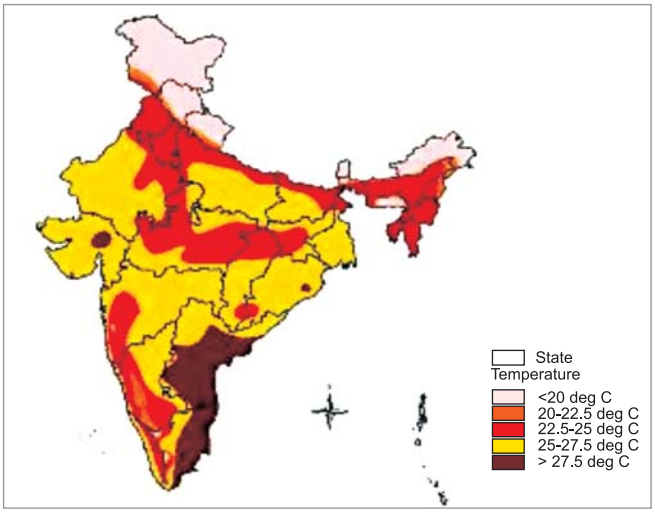
Altitude

Mosquitoe species prefer to establish at various heights where optimum ecological requirements which favour their survival are met. Anopheline species are reported from all parts of India from 1000m below the mean sea level in mines to 4500 m above the mean sea level. Survey of India map showing 12 categories of altitude was used. Altitude up to 4500 m was considered in the study, as at an altitude above it mosquito survival is greatly reduced due to low temperature.



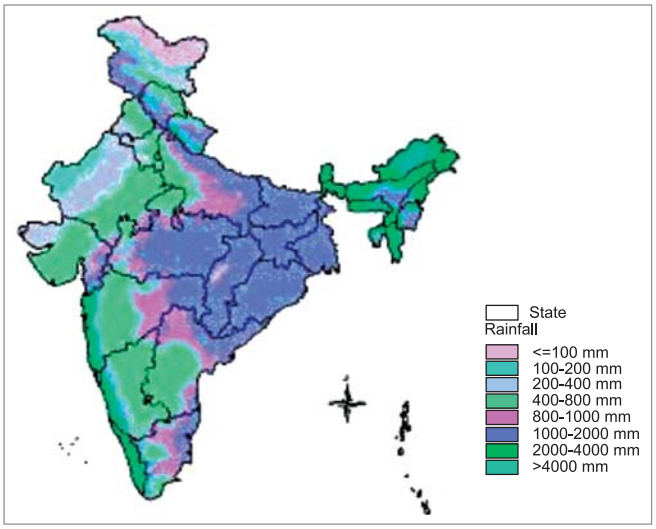
Temperature

Mosquito species have different temperature threshold levels. In addition to survival and longevity, duration of sporogony in mosquitoes is also temperature dependent. Temperature lower than 20°C prolongs the duration of sporogony in mosquito beyond 30 days *i.e.* more than the average life span and hence active malaria transmission does not take place. At extreme temperatures longevity of mosquitoes is drastically reduced. A temperature map consisting of five categories—<20°C, 20-22.5°C, 22.5-25°C, 25–27.5°C and > 27.5°C was digitized.



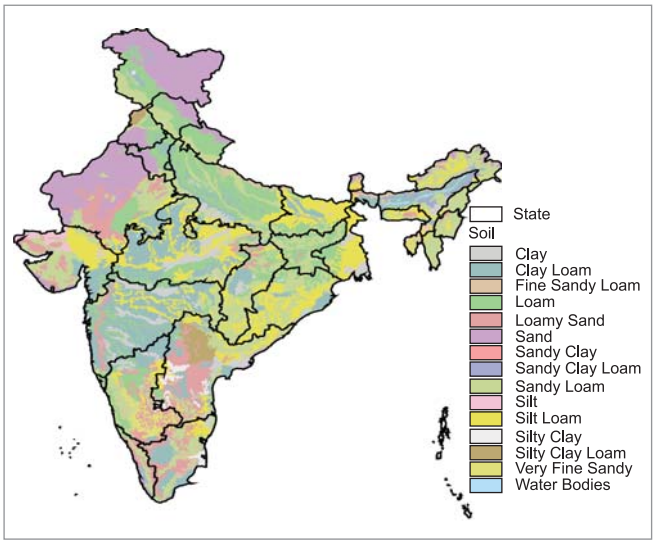
Rainfall

Mosquito species breed in a variety of water-bodies, *e.g.* irrigation channels, pools, ditches, rain water collections, streams, shallow margins, domestic containers, tree holes, creeks, *etc*. For most of the species, number of breeding sites is proportional to amount of rainfall and its pattern. Extreme conditions restrict mosquito proliferation–high rains cause flushing of breeding sites killing eggs and immature stages and low rains reduce number of breeding sites. Ten categories of rainfall ranging from <100mm to > 3200mm were considered.



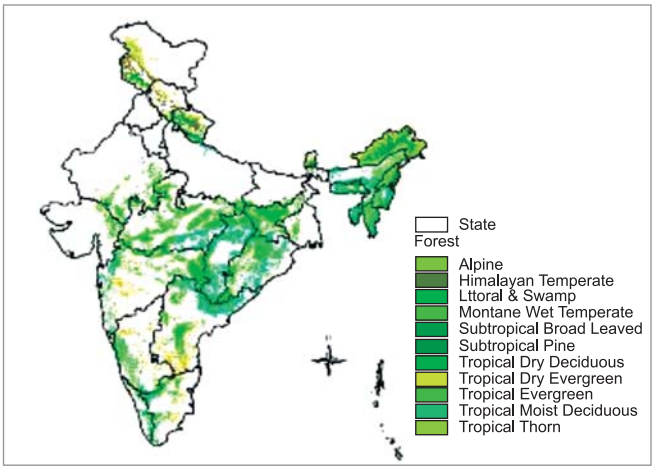
Soil

Soil is an important factor, as its drainage capacity determines internal hydro-mechanics. A soil texture map consisting of 14 categories was digitized. Soil texture guides many characteristics *e.g.* water retention capacity, water availability, drainage conditions, *etc.* Impermeable soil allows water stagnation and creates ground for mosquito breeding. On the other hand porous soil is devoid of stagnant water-bodies making it unsuitable for anopheline breeding.



Forest

For species like *An. dirus* and *An. minimus*, the home land is deep forest and forest fringe areas respectively. Similarly there are other species which are forest loving. Out of all climatic variables, rainfall plays a vital role in forest classification. A forest cover map with 11 classifications was used.



Algorithm for Identification of Favourable Range of Ecological Parameters and Integration of Thematic Maps

Reported distribution of species was taken as baseline information. From each reported area, distribution location was mapped on each thematic maps to decipher the ecological conditions at that point. A matrix was formed to identify the favourable range for each ecological parameter. Of the 50 reported locations, 30 representing various geographical locations were identified on thematic maps to decipher the corresponding parametric values. The remaining 20 reported locations were used for validation.

The range of each parameter was evaluated and for integration of themes a three dimensional space was considered, where the location of each value of each parameter on the ground was taken as the third value and favourable areas were extracted out. The

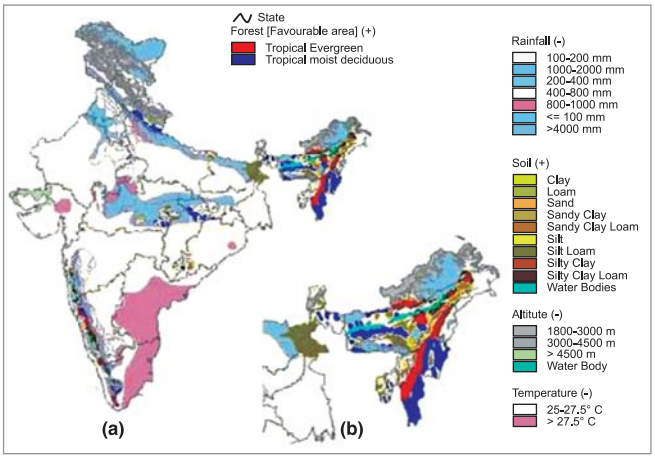


Fig. 1 (a and b) : GIS predicted favourable areas for *An. minimus* in India, shown in red and blue colors depicting two types of forest covers, tropical evergreen and tropical moist deciduous respectively (a) Distribution in India and (b) Distribution in northeastern states.

resultant map after integration of thematic maps using GIS shows the areas favourable for *An. minimus* (Fig. 1).

GIS predicted favourable areas were not only found in the northeast but in other states also *viz* Bihar, Uttar Pradesh, Madhya Pradesh, Orissa, Chattisgarh, *etc*. where this species was recorded earlier. In addition, some new areas favourable for this species were found in Kerala, Maharashtra, Himachal Pradesh and Sikkim (Fig. 1).

Field Validation

To validate GIS predictions for *An. minimus* occurrence, surveys were conducted in nine locations in four states (Table I) selected both from reported and non-reported areas in the north and the northeast and from both favourable and non favourable areas. In the northeast, a stretch of 900 kms was covered (Fig. 2).

An. minimus was found in all locations predicted favourable by GIS analysis. In Banbasa, district Champavat, Uttarakhand where the species had been reported to have disappeared after the 1950s, 7 validation surveys were conducted in different seasons and *An. minimus* immature stages or adult was reported in all the surveys. In district Dhubri in Assam malaria is reported high but *An. minimus* was not found in recent surveys. The GIS reported precisely favourable pocket area in Dhubri and the species could be collected. Thus the GIS validation surveys have established the reappearance of *An. minimus* at Banbasa and its presence for the first time in Dhubri. Blind surveys were also conducted by an independent team both in favourable and unfavourable areas. In favourable areas the species could be located but in unfavourable area on the border of Karbi Anglong, the species was not found.

Using GIS, the percentage favourable area for the distribution of *An. minimus* in different states was estimated. It showed that most of the area in the northeastern states is favourable for *An. minimus*. In Mizoram, favourable area is nearly 90.61%, in Manipur nearly 70%, and in Nagaland, Tripura and Assam nearly 35, 33 and 25 % respectively. In other states it is less than 10% except in Kerala. There are several favourable areas in Kerala and Maharashtra, but there have been no surveys and therefore, there is no confirmation for the presence of *An. minimus*.

Furthermore, the favourable corridors based on the above parameters for the distribution of *An. minimus* were also stratified as high, medium and low categories (Table II) based on type of forest, altitude, temperature range and the amount of rainfall. The species population is likely to be most stable in areas where ecological conditions are highly favourable.

Table I: Validation of *An. minimus* in GIS predicted areas by ground surveys

	Collection site/ district/ state	Period of survey	MHD** of *An. minimus*	Larval density#	GIS predicted
1	Banbasa, Uttarakhand **(Reappearance of species)**	May,2001 Jul, 2001 Aug, 2001	0.25 0.53 0.73	0 0.02 0.03	Favourable
2	Jalpaiguri*, West Bengal	Oct, 2001	1.7	0.08	Favourable
3	Dhubri, Assam **(First report)**	Oct, 2001	0.91	0.06	Favourable
4	Kamrup*, Assam	Oct, 2001	21.8	1.4	Favourable
5	Barpeta*, Assam	Oct, 2001	not done	0.18	Favourable
6	Burnihat*, Meghalaya	Oct, 2001	1.16	not done	Favourable
7	Shillong*, Meghalaya	Oct, 2001	0.33	not done	Favourable
8	Darrang*, Assam	Jun/Jul,200	04	not done	Favourable
9	Goalpara*, Assam	Sep,2001	21	not done	Favourable
10	Karbi Anglong **(Species was not found)**	Oct,2002	Zero	-	Non Favourable

* Areas already known for its presence
** MHD-Man hour density: Number of mosquitoes collected per man per hour
# Larval density: Number of larvae per dip

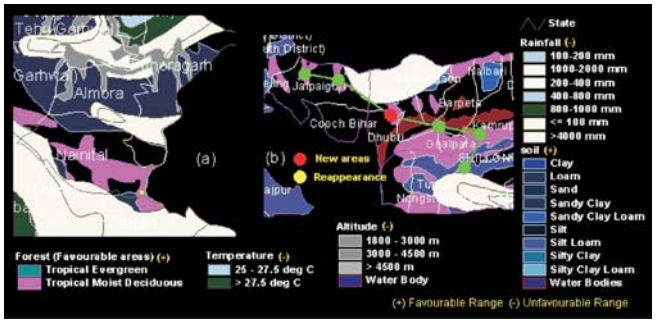


Fig. 2 Validation spots in areas of *An. minimus* distribution predicted by GIS (a) Uttarakhand, (b) Northeastern states. ● (Red)—areas where the species was reported for the first time, ● (yellow)—areas where species has reappeared after a long gap of years and ● (green)—survey spots in non-favourable areas.

Table II: Stratified favourable corridors for *An. minimus*

Favourabe	Altitude (m)	Rainfall (mm)	Temperature (deg. C)	Forest cover
High	0-600	2000-2800	22.5-25	Evergreen
Medium	600-900	2800-3200	20-22.5	Moist deciduous
Low	900-1800	3200-4000	<20	Moist deciduous

Micro Level GIS Study

The study was done at in Sonitpur in Assam which is located between 26° 24 ' and 26° 59' N latitude and 92°18' and 93°48' E longitude covering a geographical area of 5103 sq. kms. The study area falls in the tropical climate belt in the Northeastern region of India. Agriculture occupies a significant place in the economy of the state and forms the major occupation of the people. Land use of the district is divided primarily among tropical semi-evergreen, moist deciduous, riverian forest, grassland, agricultural land and tea garden. Moist deciduous forests dominate the forest cover in the districts. Out of six major vectors in India three vectors *i.e. An. minimus, An. dirus and An.fluviatilis* are responsible for malaria transmission in Assam.

Preparation of Thematic Maps

Thematic maps of altitude in the scale 1:1,000,000m; soil boundary with physical classification in the scale of 1:2,000,000; forest boundary 1: 250,000; temperature based on min/max average temperature (30 years normal) and average rainfall (30 years normal) in mm/cm for meteorological stations were procured. These maps were generated from series of National Atlas and Thematic Mapping Organizations and Survey of India sheets. Whereever possible, maps were updated using 1:50,000 scale thematic maps. District boundary in the scale of 1 : 50,000 was overlaid to extract district wise information.

Image Processing Methodology

RS images for the year 2002 and 2006 were procured through Defense Research Lab (DRL), Tejpur and North East Space Application Centre (NESAC). Images for 2006, received from NESAC were classified. Images IRS 1D and P6 - LISS III for 2008 were also procured and analyzed for further validation of GIS findings. Forest maps were updated using remote sensing images for the year 2002, 2006 and 2008. Different images were joined together to get one single false color composite (FCC). Boundary of the district Sonitpur was overlaid on FCC image and subset of the district was extracted (Fig.3). Unsupervised image classification was done and a base layer with four LULC classes of semi-evergreen; moist deciduous; scrubs and grassland and non-forest area was generated.

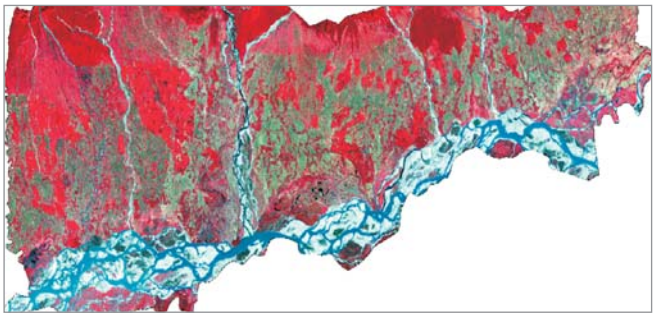


Fig. 3: Subset FCC satellite image of Sonitpur districts

For this study Arc View 9.3 and ERDAS 9.3 software were used for GIS analysis and satellite image processing.

Field Validation for Image Classification

Total six field survey were carried out using Garmin handheld GPS instrument for ground truth data collection in Sonitpur district. Daily survey track route and way points were marked and visual land use/land cover information was also recorded. A total of 7 villages belonging to 4 PHCs namely Dhekiajuli, Balipara, Rangapara and Gohpur of distt. Sonitpur were selected for survey in May, 2007. Main objective of this survey was to validate unsupervised classification of the image. Track points and way points were overlaid on the satellite image and the validation of land use/land cover classes was done for the classified satellite image; the errors were rectified accordingly.

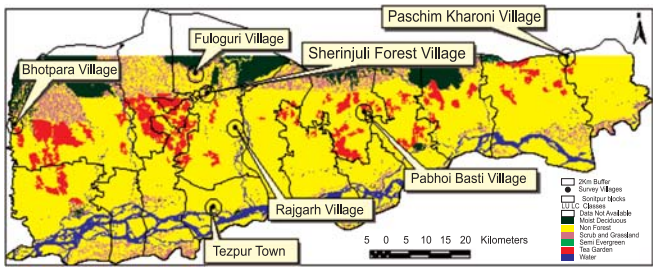


Fig 4: Validation and collection of entomological and parasitological data

GIS Mapping of Vector Distribution

After updation of forest cover map using IRS classified data, integration of favourable areas in each theme *i.e.* soil, forest cover, rainfall, temperature and altitude for each vector resulted in mapping of favourable areas for *An. minimus, An. dirus and An. fluviatilis.*

An. minimus is characteristically the species of hill and foothill but is also found in valleys even at low altitudes. It has been reported up to an altitude of about 1600m. The habits and habitats of *An. minimus* are similar to that of *An. fluviatilis* and has

overlapping areas. For mapping of distribution, forest cover was taken as the base map with evergreen moist deciduous and montane wet temparate forest as favourable areas. Areas having >1800 m altitudes, temperature > 25°C and rainfall <900 mm and >4000 mm were considered non favourable. The resultant map shows the area favourable for *An. minimus* (Fig. 5).

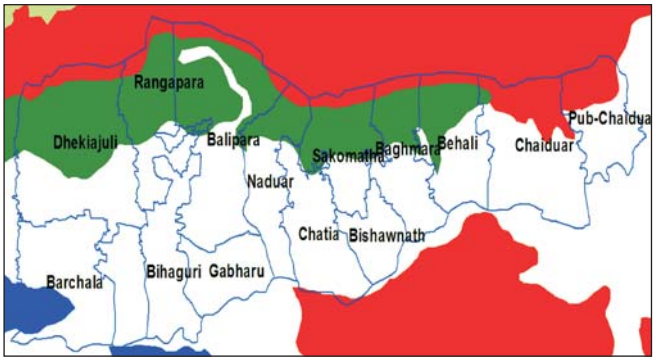


Fig. 5: GIS based distribution of *An. minimus* in Sonitpur. Red, blue and green colour shows favourable areas under different forest classes

It may also be pointed out that in Sonitpur maximum deforestation has occurred and tea gardens have increased (Fig. 6)[19]. In the deforested areas, tea gardens have been developed which support breeding of *An. minimus*.

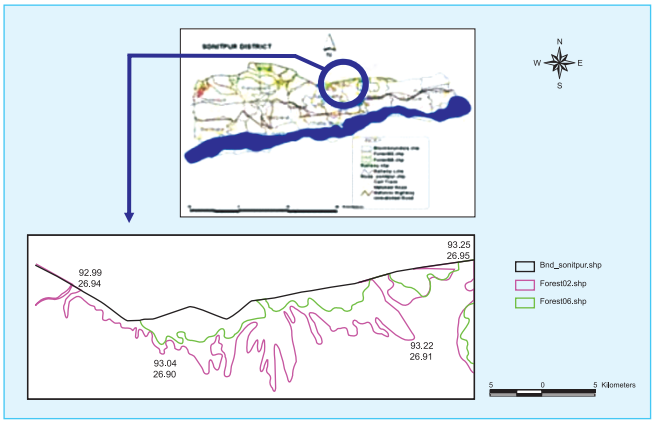


Fig. 6: Deforestation in Sonitpur from 2002 to 2006.

Similarly distribution of *An. dirus and An. fluviatilis* was mapped and the distribution of each vector was overlaid and integrated.

An. dirus is a wild mosquito and transmit malaria in deep forested areas. The species breeds in pools and other stagnant water in the forest. *An. dirus* is found in deep forested areas and these are associated with high rainfall.

An. fluviatilis is an efficient vector of malaria in hills and foothill areas of the country along with irrigated tract of Deccan plateau. It transmit disease along with *An. culicifacies* in U.P. terai and Orissa and with *An. minimus* in northeastern region. It prefers to breed in slow moving water such as streams, field channels, seepage channels of dams and irrigation with grassy shades. It breeds in shallow wells when usual breeding sites are washed out by heavy rains.

Validation of GIS Mapping

For validation of GIS mapping survey was conducted in October 2008 in Sonitpur. Out of nine villages surveyed, 4 were in GIS predicted favourable areas and five in non favourable areas. The collection of species completely reconciled with the GIS prediction.

Further, surveys were conducted in May, August and November, 2009 in favourable and non favourable areas. Total 8 villages were surveyed in favourable areas and *An. minimus* was reported in 7 villages. In one village survey could not be done properly due to difficult terrain. Maximum numbers of species were found in August survey. In 14 villages in non-favourable zone no *An. minimus* could be collected in all the three surveys.

Out of eight villages in favourable areas *An. dirus* was collected (total of 39 specimens) in five villages. Maximum specimens were found in surveys during August followed by May and November 2009. In non favourable zone no specimen could be located.

Seven *An. fluviatilis* specimens were found in 4 out of 8 villages surveyed in favourable zone. However, in 14 villages of non favourable zone, no specimen was collected.

Identification of Malaria Free Area

Distribution of maps of each vector namely *An. minimus, An. dirus* and *An. fluviatilis* was overlaid. Areas with no vector distribution were designated as malaria safe areas. Entomological and parasitological data collected from the field with GPS locations were used for validation of findings. Parasitological data indicate that malaria is high in PHCs falling in GIS predicted favourable zone.

Correlation of Epidemiological Parameters with Land Classification

Scientists of National Institute of Malaria Research also carried out the entomological (hand catch, night collection) and parasitological(active blood smear collection) data collection. PHC wise parasitological data till 2008 and entomological parameters were collected during the surveys. A significant correlation was found between tea gardens and API and it was also revealed that due to deforestation *An. dirus* was replaced by *An. minimus* and *An. fluviatilis*.

Malaria data were obtained from District Malaria Office and PHC wise correlation of area under different land use classes and epidemiological indices such as API, SPR, SfR, *etc.* were estimated to identify the land use congenial to malaria incidence. Investigations revealed a significant positive correlation of Pf% with semi evergreen and moist deciduous forest .

Decision Support

As indoor resting of malaria vectors was not encountered, indoor spray was not recommended. Hence, it is recommended that appropriate cost effective malaria control strategy should be developed for the regions by the National Vector Borne Disease Control Programme. The strategy may give emphasis that in malaria risk free areas strengthening of chemotherapy and IEC activities may be required. Along with these, personal protection and long lasting nets must be promoted to control the disease in malarious areas.

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This write-up has been contributed by Dr. Aruna Srivastava, Emeritus Medical Scientist and Dr. B.N. Nagpal, Scientist E, National Institute of Malaria Research, New Delhi.

ABSTRACTS

Some Research Projects Completed Recently

Comparison of arthroscopically assisted anterior cruciate ligament reconstruction using bone patellar tendon bone graft and semitendinoses autograft for the management of symptomatic anterior cruciate ligament in ACL deficient Knees – A clinical study

Knee inuries are extremely common now a days and their incidence has been rising in the past few decades. Majority of these injuries are sustained during sports activities and road traffic accidents. Anterior cruciate ligament (ACL) injuries are frequently neglected and treated in plaster of brace and the need of reconstruction in frequently required. The present study aimed to address the prime controversy surrounding the graft selection and evaluate the arthroscopic treatment protocol for the reconstructed ACL.

A total of 565 patients with the diagnosis of ACL tear formed the study group. Out of these, 450 patients were males and 115 were females (3.9:1). The dominant right side was involved in most of these patients. The age group affected was between 13 and 48 years (mean 26 years). The majority (356) patients were between 21 and 30 years. Road traffic accident (403 patients) was the most common cause of injury to ACL. Sports injuries form next common cause (160 patients of ACL tear).

A total of 375 patients were treated using transfix (cross pin) technique with quadrupled hamstring autograft and 290 patients using bone patellar tendon bone (BPTB) graft with interference screw. All the patients were randomly selected and operated using standard techniques. Thirty two patients had associated chondral defects and 310 patients had associated meniscal tears (200 medial meniscal tear and 110 lateral meniscal tear) which were managed armroscopically. Patients were regularly followed up for a minimum of one year. Mean IKDC score was 76 in BPTB group and 74 in hamstring group which showed that there is no significant clinical difference in these two groups in long run.

In the hamstring group tunnel widening occurred to an average of 2mm (1.5mm – 4mm) at the end of one year which had no clinically significant effect on IKDC scores or the symptoms of

the patients. Misdirected transfix pins occurred in 10 patients due to vertical femoral tunnel/high moon position of the reconstructed ACL. Nitinol wire was broken in 3 cases due to improper inventory. Anterior femoral tunnel and anterior tibial tunnel occurred in 12 cases each during initial phase. In BPTB group anterior knee pain was the most common symptom. The graft site morbidity is thus the most important problem encountered in BPTB group. The posterior blowout and the anterior femoral tunnel occurred in 16 and 17 patients respectively due to failure to identify the over the top position. Graft tunnel mismatch occurred in 43 patients which were managed using additional/alternative fixation. To summarize, ACL reconstruction using arthroscopic assisted technique is well established and successful. The semitendinosus autograft and BPTB autograft have similar result in long run. However, anterior knee pain and graft site morbidity is an important problem with BPTB graft.

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Neurogenesis in the subventricular zone and neocortex of adult rat: Interactive effects of excitotoxic injury and dietary restriction

The study was carried out on Wistar strain male albino rats in the age group of 3-4 months (weighing 150—200g) to investigate whether dietary restrictions can enhance the proliferation and differentiation of multipotent stem cell precursors in the forebrain subventicular zone (SVZ) and neocortex to neurons and improves regenerative capacity in the adult brain following excitotoxic brain injury.

It was found that short term intermittent fasting dietary regimen (DR) have profound effects on brain function and vulnerability to injury and disease. It was also found that DR can stimulate the production of new neurons from stem cells (neurogenesis) and can enhance synaptic plasticity, which modulate pain sensation, enhance cognitive function, and may increase the ability of the brain to resist aging. Moreover the beneficial effects of DR appear to be the result of a cellular stress response stimulating the production of proteins that enhance neuronal plasticity and resistance to oxidative and metabolic insults. They include neurotrophic factors such as BDNF and NT-3, protein chaperones such as HSP-70, and neuronal plasticity regulators. Enhanced neurogenesis appeared to occur in concert with ongoing neurodegeneration after the administration of a low dose of pilocarpine to adult rats.

Thus, excitotoxicity in adult rats was found to initiate a complex set of events that have long-term consequences for structure and function of brain tissue. These results support the general hypothesis that the adult rats CNS is capable of regeneration in response to neuronal injury or degeneration under specific conditions and intermittent fasting DR regimen shows beneficial effects by further enhancing neurogenesis in these germinative zones.

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Publications

1. Sharma, S. and Kaur, G. Intermittent dietary restriction as a practical intervention in aging. *Ann N Y Acad Sci 1114*: 419, 2007.
2. Sharma, S. and Kaur, G. Dietary restriction enhances kainate-induced increase in NCAM while blocking the glial activation in adult rat brain. *Neurochem Res 33*: 1178, 2008.
3. Kumar, S., Parkash, J., Kataria, H. and Kaur, G. Interactive effect of excitotoxic injury and dietary restriction on neurogenesis and neurotrophic factors in adult male rat brain. *Neurosci Res 65*: 367, 2010.

Role of xenobiotic metabolizing gene polymorphisms in lung cancer susceptibility

Study was carried out to analyse whether genetic polymorphisms in phase I (CYP1A1, CYP2E1 and CYP2D6) and phase II (GSTM1, GSTT1, GSTP1) genes confer an increased risk to develop lung cancer, elucidate the role of these genetic polymorphisms, either singly or combined, as genetic susceptibility markers in lung carcinogenesis, and to determine whether any of the specific genotypes has any association with any lung cancer subtype phenotype. Efforts where also made to determine the survival status in relation to common genetic polymorphisms affecting phase I and phase II enzymes in lung cancer patients. The study was conducted on 211 lung cancer patients as well as 211 healthy controls matched for age and geographic origin.

On genotype analysis, the frequencies of variant genotypes of the studied genes could be determined and the possible association with lung cancer susceptibility could be derived. The data generated from the study clearly indicated that the genetic polymorphisms in low penetrance genes coding enzymes involved in xenobiotic metabolism, DNA repair and cell cycle regulation could be important modifiers of the susceptibility to lung cancer in the Kerala population. Besides modulating the susceptibility, inter individual genetic variation can also contribute to the prognosis and outcomes of the cancer. These common polymorphisms acting in concert with lifestyle factors, may thus account for a large proportion of the population susceptible to lung cancer. Hence, specific predisposition genotypes identified in the present study could be considered as biomarkers which could be used for the early detection of high risk individuals. The present study could identify new biomarkers, which in turn could identify susceptible individuals or subgroups that are at high risk of developing lung cancer. The results of this study indicated that the inheritance of multiple unfavourable genotypes especially activating genes, is a crucial predisposing factor for the development of lung cancer from cigarette smoking.

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Publications

1. Sreeja, L., Madhavan, J. and Ankathil, R. A Multigenic approach to predict risk and survival among Indian lung cancer patients. *J Thor Oncol. 2 (suppl. 4)*: S566, 2007.
2. Sreeja, L., Syamala, V., Hariharan, S., Syamala, V.S., Raveendran, P.B., Sivanandan, C.D., Madhavan, J. and Ankathil, R. Glutathione S-transferase M1, T1 and P1 polymorphisms: Susceptibility and outcome in lung cancer patients. *J Exp Ther Oncol 7:* 73, 2008.

Development of laser spectroscopy techniques for early detection and follow-up of therapy in breast malignaricy

A feasible study of identification of chromatographic markers by HPLC-LIF for the normal, benign and malignant breast tissues as well as blood samples were investigated. Typical chromatograms of normal, benign, malignant and post-treatment breast tissues homogenates as well as the blood samples exhibited significant differences. PCA of baseline corrected as well as derivative spectra of tissue extract based on score of factor 1 and 2 gave good discrimination between normal and malignant conditions whereas the benign and post-treatment chromatograms were overlapping between normal and malignant conditions. The possibility of classifying tumors with or without residual tumor in after-chemotherapy tissues by Raman spectroscopy was demonstrated on a limited number of cases.

It is thus concluded that optical spectroscopic studies using LIF and Raman demonstrated the feasibility of discriminating healthy and diseases tissues. Raman spectral profiles of normal and pathological (benign and malignant) breast tissues suggest predominance of lipids and proteins, respectively which correlates quite well with our understanding of neoplastic process.

Mean Raman spectra of normal, benign and malignant breast tissues exhibited significant differences. The spectral features of normal and pathological conditions indicate lipids and proteins, respectively. Principal component analysis (PCA) of 1400 – 1750 cm-1 region with 9 factors gave best discrimination. Training sets were developed using 36 normal, 35 malignant and 21 benign spectra based on scores of factor 1 and histopathological certification. These models were evaluated using blinded samples. A good correlation between spectroscopic and histopathological diagnosis was observed.

The emission spectral profiles obtained on 325 nm excitation, of normal, benign and malignant breast tissue exhibited significant differences. A complete reversal in peak intensities of 390 nm and 460 nm is observed for normal and pathological tissues. Among pathological tissues, major differences are sharper 390 nm and weaker 460 nm peaks characterizing benign tissues in comparison to malignant tissues. Curve analysis of fluorescence spectra indicated higher levels of collagen in both types of abnormal breast lesions. Among the abnormal breast lesions, higher content of total NAD(P) H, especially bound NAD(P)H seems to be a

characteristic intrinsic fluorescent marker of malignant tissues whereas higher values of bound to free BNAD(P)H ratio in benign tissues. PCA was carried out in two approaches: unsupervised and supervised methods. Both the approaches gave good discrimination.

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Publications

1. Chowdary, M.V.P., Kumar, K.K., Kurien, J., Mathew, S. and Murali Krishna, C. Discrimination of normal, benign and malignant breast tissue by Raman spectroscopy. *Biopolymers 83:* 556, 2006.
2. Murali Krishna, C., Kurien, J., Mathew, S., Rao, L., Maheedhar, K., Kumar, K.K. and Chowdary, M.V.P. Raman spectroscopy study of breast tissues: A review. *Expert Rev Mol Diagn 8*: 149, 2008

Homocysteine and stroke

The present study was designed to determine plasma homocysteine levels in a cross-section of adult population and find out its relationship with age, gender, vitamin B6, B12, folic acid, diets and other risk factors of stroke. It was also planned to study the relationship of homocysteine with ischaemic and haemorrhagic stroke. Altogether 970 people above the age of 35 were taken up for the study with a male and female ratio of 51.3 : 48.7. The mean homocysteine level in male was found to be 20.35 ± 8.74 and that for female 16.36 ± 7.27 mmol/litre. Mean homocysteine was also found to be high amongst the vegetarians (23.53 ± 15.43 mmol/litre) than that of the non-vetgetarians (18.32 ± 8.10 mmol/litre). There was an inverse relationship between plasma homocysteine levels and vitamin B6, B12 an folic Acid. Results of multivariate analysis showed positive relationship between homocysteine and established risk factors of stroke *e.g.* systolic and diastolic hypertension and BMI (Obesity), creatinine and uric acid.

Case control study with 110 ischaemic stroke patients and equal number of age and gender matched control did not show any significant difference in the homocysteine levels between the two groups. Mean homocysteine levels in the ischaemic stroke was 19.45 ± 8.68 mmol/litre and that of the control was 18.69 ± 9.04 mmol/litre. Odds that a person who has hyperhomocysteinemia having ischameic stroke is 1.118 times more than a person with normal homocysteine. A similar study in 110 patients of haemorrhagic stroke with equal numbers of age and gender matched control revealed significantly high homocysteine level in the haemorrhagic stroke patients (23.53 ± 12.03 mmol/litre) compared to the control group (18.13 ± 8.84 mmol/litre). The odds that a person who has high homocysteine (>16 mmol/L) will have haemorrhagic stroke is 1.887 times more than that of a person with normal homocysteine.

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ICMR NEWS

The following meetings of various technical groups/committees of Council were held in May 2010.

Meeting of Scientific Advisory Group (SAG)

SAG of the Division of Publication and Information	May 13, 2010 (at New Delhi)

Meetings of Project Review Committees (PRCs)/Expert Groups (EGs)/Task Forces (TFs)/ and Other Meetings held:

PRC on Trauma, Accidents and Injuries	May 3, 2010 (At New Delhi)
RFC Meeting on Data Repository and Business Intelligence	May 3, 2010 (at New Delhi)
PRC on ICMR-INSERM Projcts	May 11, 2010 (at New Delhi)
EG on Toxic Element of Toys	May 14, 2010 (at New Delhi)
Public Consultation on ICMR-DBT Guidelines for Stem Cell Research and Therapy	May 14, 2010 (At Dibrugarh)

Meeting of Childhood Obesity in India and Multicentre Study on Its Measurement and Determinants	May 19, 2010 (at New Delhi)
PRC on Oral Health	May 20, 2010 (at New Delhi)
PRC on Cardiovascular Diseases	May 21, 2010 (at New Delhi)
TF on Nanomedicine	May 26, 2010 (at New Delhi)

Participation of ICMR Scientists in Scientific Events

Dr. K.D. Ramaiah, Scientist E, Vector Control Research Centre, Pondicherry, participated in the Informal Meeting of WHO to Review Lymphatic Filariasis Elimination Programme Projects Report 2000-2009 and Lymphatic Filariasis Plan for 2010-2020, at Geneva (May3-8, 2010).

Dr. V. Gopalakrishna, Scientist D, National Institute of Virology (NIV), Pune, participated in the XXVIII Annual Meeting of the European Society for Paediatrics Diseases, at Nice (May 4-8, 2010).

Dr. B. Sesikaran, Director, National Institute of Nutrition (NIN), Hyderabad, participated in the Symposium on Advances in Nutrition Research and Technology: Perspectives in Asia, at Singapore (May 5-6, 2010).

Dr. R. Ramakrishana, Scientist E and Dr. Tarun Bhatnagar, Scientist C, National Institute of Epidemiology (NIE), Chennai, participated in the Review Meeting of the Collaborative Project with Tufts University, at Boston (May 9-24, 2010).

Dr. J.M. Deshpande, Director, Enterovirus Research Centre, Mumbai, participated in the Polio Research Committee Meeting, at Geneva (May 10-11, 2010).

Dr. T. Ramamurthy, Scientist F, National Institute of Cholera and Enteric Diseases (NICED), Kolkata, participated in the X International Advanced Course on Vaccinology in Asia-Pacific Region, at Seoul (May 10-15, 2010).

Dr. R.S. Paranjape, Director, National AIDS Research Institute (NARI), Pune, participated in the (i) Cross-RPC Conference on SRH and HIV; and (ii) Evidence for Action Consortium Meeting, at London (May 17-18, and 19-21, 2010 respectively) He and Dr. L.E. Heena, Scientist, B Tuberculosis Research Centre (TRC), Chennai, also participated in the Bio-Regional Asia pacific Workshop on HIV Drug Resistance Genotyping, at Ho Chi Minh City (May 25-28, 2010).

Dr. S.L. Chauhan, and Dr. K.V.R. Reddy, Scientists E, Dr. A.H. Bandivdekar and Dr. Jayanti Mania- Parmanik, Scientists D, National Institute for Research in Reproductive Health (NIRRH), Mumbai and Dr. Smita Kulkarni, Scientist D, NIV, Pune, participated in the International Conference on Microbicide- 2010, at Pittsburgh (May 22-25, 2010).

Dr. Beena E.Thomas, Scientist B, TRC, Chennai, participated in the V International Conference on HIV Treatment Adherence, at Miami (May 23-25, 2010).

Dr. N. Arunachalam, Scientist F, Centre for Research in Medical Entomology, Madurai, participated in the International Symposium on Ecohealth Ecobiosocial Research on Dengue: The Asian Prespectives, at Dhaka (May 23-26, 2010).

Dr. Poonam Salotra, Scientist E, Institute of Pathology, New Delhi, worked in the Laboratory of Dr. Hira Nakhasi, Director, Centre for Biologics Evaluation and Research, Bethesda for a Collaborative Project on Vaccine Development for Kala-azar (May 26-June 11, 2010).

Dr. Neena Valecha, Scientist F, National Institute of Malaria Research (NIMR), New Delhi, participated in the III Meeting of Scientific Advisory Committee on Antimalarial Policy Access, at Geneva (May 31-June1, 2010).

Workshops/Training Courses

Dr. Suman Kanungo, Scientist B, NICED, Kolkata, participated in the XI advanced Vaccinology Course at Annecy (May 9-21, 2010).

Dr. P.N. Yergolkar, Scientist D and Dr. R.S. Jadi, Scientist C, NIV, Pune, participated in the Regional Consultation of Virologists from South East Asia Regional Measles and Rubella Laboratory Network, at Bangkok (May 13-14, 2010).

Dr. R.C. Dhiman, Scientist F, NIMR, New Delhi, participated in the Training Course on Climate Information for Health at Palisades (May 17-28, 2010).

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