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EPIDEMIOLOGY AND SURVEILLANCE OF JAPANESE ENCEPHALITIS IN TAMIL NADU

Japanese encephalitis (JE) is a non-contagious, mosquito-transmitted virus disease mainly affecting children below 15 years of age. Severe epidemics have occurred in the past and there is evidence to suggest that the disease is spreading to new areas. Kerala, which was so far free from JE, experienced the first outbreak in 1996, and since then sporadic cases are being reported. The epidemiology of JE is complex due to a complex transmission cycle of the virus involving a variety of vertebrate and invertebrate hosts. Although considerable information on the disease is available, there are still gaps in knowledge about the epidemiology which need to be understood in order to develop sound surveillance systems for forecasting epidemics and control strategies. The Centre for Research in Medical Entomology (CRME), Madurai, has addressed these issues for over a decade. In this article, it is intended to provide an update of research to understand the epidemiology of the disease in Tamil Nadu, quantify transmission, and develop surveillance systems, which may serve as paradigms for other endemic areas.

Epidemiology

Human infections

In JE, there is always a preponderance of subclinical infections and therefore, reporting only overt cases underestimates the total level of virus transmission in an endemic area. Subclinical infections can be quantified by estimating seroconversion rates in susceptible age-groups of children.

South Arcot (now Cuddalore) and Thanjavur districts, present two distinct epidemiological patterns. South Arcot is relatively dry but, an extensive epidemic occurred in 1981[1] and since then the disease has remained endemic with many cases of encephalitis occurring regularly each year. Records of the past 10 years show an average annual incidence of 4.7/100,000 population. Thanjavur district is the major rice growing area in Tamil Nadu, and has provided favourable breeding conditions for JE vectors. But, paradoxically there are no outbreaks of JE and the encephalitis case incidence is very low (0.4/100,000). A study between 1989-91 in rural children of the age group 5-9 years in Nallur primary health centre (PHC) in South Arcot district showed that the seroconversion rates for flaviviruses in successive years were 43.7; 67.6 and 47.9%; the seroconversion being predominantly due to JE and minimally to West Nile virus. Relatively high dengue virus activity was seen only in 1991. The estimated ratio of overt : inapparent infections was 1 : 270[2]. A 2 year (1991 and 1992) prospective study of comparable age-groups in 3 PHCs in Thanjavur district showed that seroconversion rates for flaviviruses were 32.1 and 25.3% with dengue virus predominating (20 and 8%). JE infection rates were

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only 2 and 5%. The apparent : inapparent infection ratio was 1 : 1774 (unpublished observations). Clearly the transmission intensity in Thanjavur district is much lower than that in South Arcot district. Reasonably assuming that the virulence of the virus is similar in both these contiguous districts, the low case incidence in Thanjavur may be due to the low intensity of JE transmission.

Complete data are not available on the dynamics of JE transmission in Thanjavur district to account for low JE transmission. However, a high cattle : pigs ratio and a high dengue infection rate observed in this district, appear to be important for a discussion on this issue. The vectors of JE are highly zoophagic mainly feeding on large domestic animals. Pigs are the amplifying hosts for JE virus while cattle act as 'brakes' for JE virus transmission. In South Arcot district, children in villages with a higher ratio of cattle : pigs showed lower seroconversion rates than in villages with lower ratios[2]. Therefore, the relative abundance of these hosts appears to be important in modulating the JE activity in an endemic area. In Thanjavur, the ratio of pigs : cattle was 1 : 400, whereas in South Arcot it was 1 : 4. This suggests that a lower pig and higher cattle density may be an important factor in limiting JE transmission in Thanjavur district.

In both years of study in Thanjavur district, dengue virus was predominant. In 1991, when dengue infection rate was 20%, the JE infection rate was 2% and in 1992 when dengue infection rate declined to 8%, the JE infection rate rose to 5%. A similar observation was made in South Arcot district also. In 1989 and 1990, the JE infection rate was 30 and 39% respectively and during these years there was no evidence of dengue virus activity. In 1991, there was a spurt in dengue virus activity and an infection rate of 16% was recorded with a decline in JE virus infection to 24%[2]. These observations suggested that dengue and JE virus infections may be mutually exclusive. Earlier workers have also observed that children exposed to dengue were in someway protected against development of JE[3,4]. However, more epidemiological data are needed to define the role of dengue in the context of low transmission in Thanjavur district.

JE incidence appears to be generally higher in males than in females (male : female ratio 2 : 1 in Kolar[5] and 1.0 : 0.8 in Nallur[2]). But subclinical infections occurred equally in both sexes, since seroconversion rates for males and females were comparable in both the South Arcot and Thanjavur districts indicating that both sexes were similarly exposed to infection. Therefore, it is important to know whether gender difference in disease incidence is real or due to some other confounding factor(s).

Mosquito infection

Vector infection rate is an important parameter for quantifying transmission potential of vectors and risk of transmission to humans. From the serological study in South Arcot district it was estimated that the probability of a child receiving an infective mosquito bite during the JE virus transmission season was between 0.5 to 0.75[2]. This implied a high transmission potential of vector population. Vector infection frequency in relation to vector abundance was studied between 1991 and 1994 in the same areas where serological study was conducted previously. Out of 5710 pools (285, 531 mosquitoes) processed, 91 virus isolations were made and 80% of them were identified as JE. Vector abundance and minimum infection rate (MIR) increased from July concurrently with the initiation of rice cultivation but MIR peaked in September followed by a decrease in October, whereas mosquitoes remained abundant until March. The decrease in MIR from October onwards coincided with the rising herd immunity in pigs. Although MIR in October (0.47) and November (0.42) were lower than in September (0.92), a comparable high risk of infection continued because of high vector abundance and human biting rates and most episodes of encephalitis occurred during these months (Fig. 1). The estimated probability of a child receiving an infective bite was 0.53 per transmission season, which reasonably agreed with the estimated probability (0.5 to 0.75) based on serological studies[6]. A similar study may help in explaining the low transmission level observed in Thanjavur district.

The JE virus is maintained in nature by a complex cycle that involves pigs as amplifying hosts, ardeid birds as reservoirs and mosquitoes as vectors. Thus, the virus is maintained mainly through biological transmission between vertebrate hosts by vector mosquitoes. Vertical transmission of JE virus in mosquitoes has been observed, but was considered of no epidemiological significance, in the belief that it occurred rarely, if at all, in nature. However, recent studies in the field by Dhanda *et al*[7], showed that vertical transmission may occur quite frequently, but its real epidemiological significance is as yet unclear. In order to understand more about vertical transmission, scientists at the CRME conducted a study between 1994-97 in Cuddalore (a high JE endemicity area) and Madurai (a very low JE endemicity area) districts. There was a clear difference in vertical transmission between the two areas. In Cuddalore district 2720 pools (136,000 mosquitoes) of adults reared from wild-caught immatures were examined for virus infection and the MIR was 2.62. The MIR showed a higher peak during the hot months (April to July) and a lower one during the cool

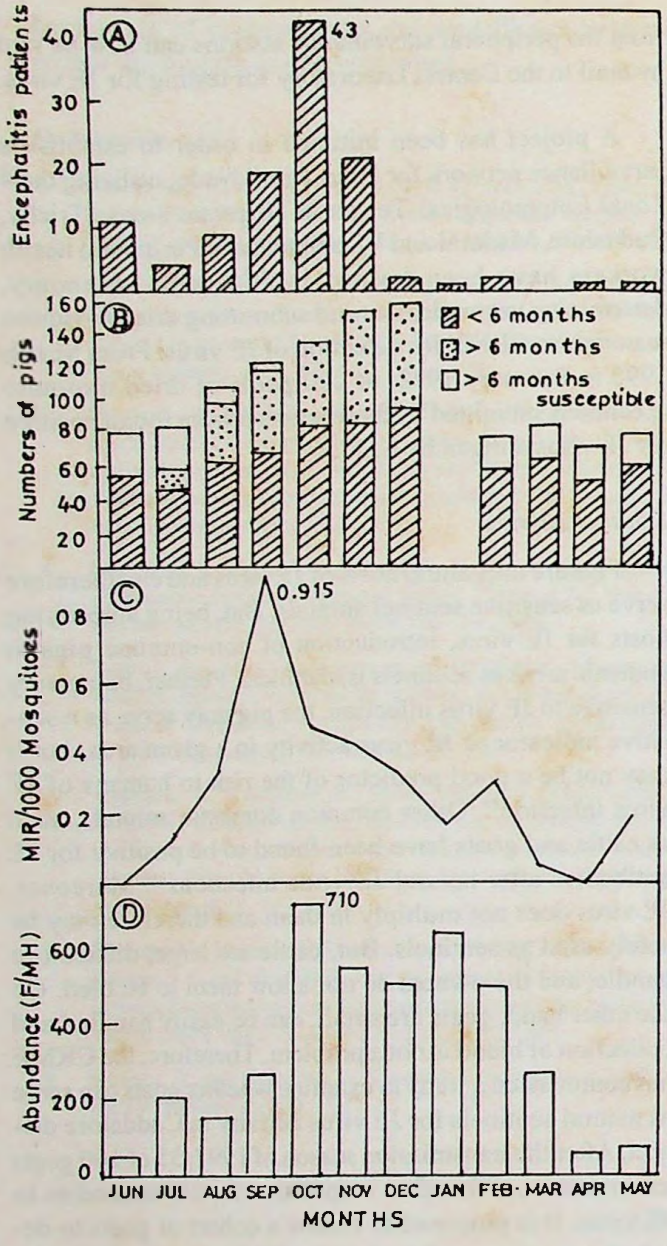


Fig. 1. (A) Encephalitis patients. (B) Pig census by age with calculated number seroconverted. (C) Vector infection. Minimum infection rate (MIR)/1,000 mosquitoes tested. (D) Vector abundance. Number of female adults collected per man hour (F/MH).

months of September to February (Fig. 2). This correlated significantly with infection in wild-caught adults. In Madurai district 968 pools (42,418 mosquitoes) were examined, and the MIR was 0.54. There was no difinite pattern in seasonal variation in infection rates (unpublished observations). In South Arcot district in general, during hot months vector density is very low and very few non-

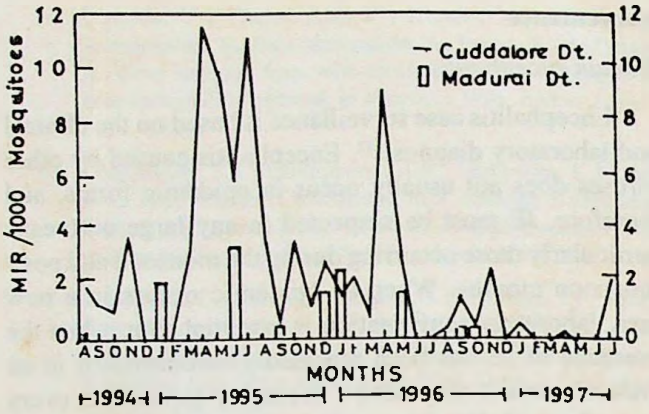


Fig. 2. Minimum infection rate (MIR) of *Cx. tritaeniorhynchus* adults reared from immatures in Cuddalore and Madurai districts.

immune pigs are available[6]. Therefore, vertical transmission may be an adaptation by the JE virus to tide over these adverse conditions.

Suspected vectors

JE virus has been isolated from 10 species of genus *Culex*, 2 species of *Anopheles* and 3 species of *Mansonia* in India[8]. However, *Culex tritaeniorhynchus* and *Cx. vishnui* have been incriminated as the primary vectors of JE. Role of Anophelines in transmission of JE virus has not been systematically studied. A 2 year study in Cuddalore district showed that abundance of *Anopheles subpictus* (the dominant species among *Anopheles* in the rice ecosystem) was much lower than that of *Cx. tritaeniorhynchus*. The density of *An. subpictus* was higher in hot months (non-JE transmission season) than in cool months (transmission season). This species fed poorly on pigs (0.6%) and humans (0.3%) and JE virus infection was not detected. In a parallel study with *Cx. tritaeniorhynchus*, 2.9% fed on pigs, 2.9% on humans, the MIR was 0.57, and the density was high during JE transmission season. Therefore, it is unlikely, that *An. subpictus* species plays a role in JE virus transmission in Cuddalore district.

In the recent Kerala outbreak, of the 12 JE virus isolations, 4 were from *Mansonia* species[9]. Previously, one isolation was made from *Ma. annulifera* in Dibrugarh, Assam[10] and one JE virus antigen positive pool was detected in Mandya district, Karnataka[11]. Four isolations from a small number of *Mansonia* species in Kerala is noteworthy. Therefore, a detailed study of the vectorial potential of *Mansonia* species (which are vectors of brugian filariasis in Kerala) for JE transmission in Kerala has been planned.

Surveillance

Human encephalitis

Encephalitis case surveillance is based on the clinical and laboratory diagnosis[12]. Encephalitis caused by other viruses does not usually occur in epidemic forms, and therefore, JE must be suspected in any large outbreaks particularly those occurring during the monsoon and post-monsoon months. When an epidemic occurs in a new area, laboratory confirmation is essential. But, when the presence of JE has been repeatedly demonstrated in an endemic area it may not be necessary to confirm every case of encephalitis. Nevertheless, for surveillance records in a new area it is essential to determine the proportion of JE among encephalitis cases. For example, in South Arcot district during the JE transmission seasons of 1993-1995, 85 patients (mostly paediatric) with acute central nervous system diseases were examined using a battery of laboratory diagnostic tests for JE. In 53 patients (62.4%) a diagnosis of JE was established. In terms of diagnostic value IgM antibody capture ELISA (MAC ELISA) on convalescent serum had the highest sensitivity (89%) and negative predictive value (NPV) (50%) followed by MAC ELISA on acute serum and CSF which had similar sensitivity (84%) and NPV (40%). The IF test to detect JE virus antigen in CSF cells was least sensitive (58%) but the distinct advantage of this test was that it could be completed in about 2-3 h[13]. The MAC ELISA can be performed using finger-prick blood specimens. Therefore, MAC ELISA and IF tests are particularly appropriate in JE surveillance programmes.

Vector surveillance

Vector abundance and vector infection are two important parameters in vector surveillance. A simple Dusk Index (DI) defined as a product of the average number of vector females per man-hour in dusk collections and proportion parous, has been developed which can be used for monitoring vector abundance. In a study in Thanjakoor, near Madurai, significant human-biting activity was not observed when the value of DI was less than 50 parous females per man hour[14]. However, DI needs further validation in different epidemiological situations.

An antigen capture ELISA for detection of JE virus in wild-caught mosquito has been recently validated in a large-scale field trial in South Arcot district[6]. This rapid screening system is particularly appropriate as a surveillance tool. Laboratory and field studies showed that JE virus antigen can be detected in desiccated mosquitoes by ELISA. Therefore dried specimens of vectors collected from the peripheral surveillance stations can now be sent by mail to the Central Laboratory for testing for JE virus.

A project has been initiated in order to establish a surveillance network for JE in Tamil Nadu, utilizing the 4 Zonal Entomological Teams in JE prone areas (Trichy, Cuddalore, Madurai and Virudhunagar). Peripheral health workers have been trained in JE vector taxonomy, determining vector density and submitting dried mosquito materials to CRME for detection of JE virus. From March 1996 to February 1998, of 200 pools of dried mosquito specimens submitted by these teams 3 were found positive for JE virus antigen by ELISA.

Sentinel animals

Pigs are the natural hosts of JE virus and can therefore serve as sensitive sentinel animals. But, being amplifying hosts for JE virus, introduction of non-immune pigs to endemic areas as sentinels is unethical. Further, being very sensitive to JE virus infection, the pig may serve as a sensitive indicator of JE virus activity in a given area, but it may not be a good predictor of the risk to humans of JE virus infection[15]. Other common domestic animals, such as cattle and goats have been found to be positive for JE antibodies after natural JE virus infections[16]. Moreover, JE virus does not multiply in them and therefore may be safely used as sentinels. But, cattle are large, difficult to handle, and the owners do not allow them to be bled. On the other hand, goats are small, can be easily handled and collection of blood is not a problem. Therefore, the CRME has commenced a study to examine whether goats can serve as natural sentinels for JE virus activity in Cuddalore district. After the transmission season of 1997, 35 of 100 goats examined were found to be positive for HI antibodies to JE virus. It is proposed to follow a cohort of goats to determine seroconversion rates in different seasons.

Conclusions

In India millions of children are exposed to JE virus infection each year in endemic areas and hundreds of them suffer from JE. JE is a vaccine preventable disease but, unfortunately there has been no national policy on vaccination against JE. This is partly because there is a lack of epidemiological data from different endemic areas which can focus on the gravity of situation. Epidemics of JE continue to occur in different parts of the country and the disease is spreading to newer areas. But, epidemic-prone states have no plans to set up effective surveillance systems which are urgently needed to monitor JE activity. It is essential that appropriate control measures are taken based on sound

knowledge of epidemiology and surveillance strategies, so that the disease can be controlled.

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This article has been contributed by Dr. A. Gajanana, Office-in-Charge, Centre for Research in Medical Entomology, Madurai.

ABSTRACTS

Some Research Projects Completed Recently

Role of some non-steroidal anti-inflammatory drugs in carcinogenesis.

The study was carried out in male weanling inbred Swiss mice to evaluate the role of non-steroidal anti-inflammatory drugs (NSAIDs) *viz.* piroxican, diclofenac and naproxen in carcinogenesis. The animals were fed the NSAIDs through diet. For mechanistic studies the doses of diclofenac, naproxan and piroxican were 25, 75 and 375 ppm; 150, 500 and 1500 ppm; and 3, 10 and 30 ppm respectively for 4 weeks. For tumour studies the highest doses of the NSAIDs were used with carcinogen Nitrosodiethylamine (NDEA) in the dose of 80 mg/kg body wt. at 4 weekly intervals thrice.

Diclofenac in a dose of 375 ppm in the diet inhibited NDEA induced lung tumourigenesis by acting at the initiation and post-initiation phases of carcinogenesis, whereas naproxen in a dose of 1500 ppm stimulated the promotional phase of carcinogenesis.

Ornithine decarboxylase (ODC), which is the rate limiting enzyme in mammalian polyamine biosynthesis required for tumour promotion and cell proliferation in various organs, was inhibited by diclofenac and piroxican, but was stimulated by naproxen indicating that this enzyme is a good marker for tumourigenesis and for agents with anticarcinogenic properties.

Prostaglandin E_2 release, which is involved in inflammation and tumour promotion, was decreased to a variable degree in plasma, liver and lung by all three NSAIDs tested; diclofenac being the strongest, and naproxen the weakest inhibitor. Diclofenac and piroxican slowed down the NADPH and ascorbate dependent microsomal lipid peroxidation *in vivo* and *in vitro*, whereas naproxen at a concentration of 10 mM stimulated the process. It seems that besides being a scavenger of free radicals, enzymatic metabolism of naproxen at higher concentration formed reactive intermediates/free radicals which exceeded its scavenging capacity. The feeding of NSAIDs to mice inhibited the process of lipid peroxidation and stimulated the tissue antioxidant defense system comprising superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and reduced glutathione. It also resulted in the decrease in cytochrome P-450 levels.

Increased activities of glutathione-s-transferase, glutathione reductase and reduced glutathione by NSAIDs may detoxify the xenobiotics more efficiently. The enhanced levels of reduced glutathione, in addition may provide protection to tissues against the damaging action of free radicals.

The enhanced tumourigenesis and ODC levels produced by naproxen requires further evaluation for its safety.

K.L. Khanduja
Department of Biophysics
Postgraduate Institute of
Medical Eduction and Research
Chandigarh

Evaluation of intra-ocular lens implantation in children.

Intra-ocular lens (IOL) implantation was evaluated in 75 eyes of 45 patients aged 14 months to 12 yr, with congenital, traumatic and developmental cataracts. Of the 75 eyes, 30 eyes were from 15 patients with congenital cataract, 15 from 15 patients with traumatic cataract and 30 from 15 patients with developmental cataract. For comparison 15 eyes (4 with congenital, 6 with traumatic and 5 with developmental cataracts) had surgery without IOL implantation (aphakic eyes). Eyes with associated systemic or ocular pathologies (other than amblyopia and strabismus) likely to affect visual prognosis later were excluded from the study.

Posterior chamber intra-ocular lenses in the range of +20 to +24 D were implanted after computerized power calculation keeping the amblyopia factor and prospective adulthood refraction in mind, and managing the school age children with low powered bifocals

IOL implantation was found to be difficult in children compared to adults. Pseudophakes were found to have distinct advantages of better quality good binocular vision compared to aphakes who suffered from problems of magnification, disorientation, cosmetic blemish, field restrictions, anisometeropia and diplopia caused by aphakic correction. Problems of poor compliance, corneal complications and costly replacements seen with contact lens use were also eliminated with IOL implantation. Placement of the IOL in the bag is close to the physiological and anatomical positions of the natural lens and hence comparatively safe.

Intra-operative problem of scleral collapse in children was managed by application of Fleringa's ring under general anaesthesia. Post-operative severe uveitis responded to frequent administration of steroid drops for 8 to 12 weeks as well as oral steroids in the first week. Pupillary capture in 80% cases of cataract was prevented by keeping the pupil mid dilated. Over 86% of patients showed early after cataract (post capsular thickening) which responded to early YAG-laser capsulotomy in children aged above 5 yr, while this complication could be handled by secondary discission within 6 months of surgery in the age group below 5 yr. Best results were obtained in 14% of patients where primary post capsulotomy/rhexis with anterior vitrectomy was done followed by IOL implantation.

It can thus be concluded that IOL implantation is a safe and effective aphakic rehabilitation method in the management of developmental and congenital cataracts as also in the case of traumatic cataract. However, the final visual outcome in traumatic cataract depends upon the amount of damage that had occurred to various ocular structures due to the injury.

Visual recovery may be further improved and rate of complications reduced by lowering of intra-ocular pressure preoperatively, use of scleral support to prevent scleral collapse, control of iritis and maintaining pupillary mobility, promt management of posterior capsular opacification along with vigorous amblyopia therapy.

Arun Baveja
D.P. Vats
Army Hospital
Delhi

ICMR NEWS

The following meetings of various technical groups/ committees of the Council were held:

Meeting of the Scientific Advisory Committee (SAC):

SAC of the Centre for Advanced Research on Standardization, Quality Control and Formulation of Traditional Remedies/Natural Products, Jammu	April 2, 1998 (at Jammu)

Meetings of the Expert Group (EG)/Project Review Committee (PRC) and Annual Review Meetings held at New Delhi:

Meeting on Situation Analysis of HIV and Drug Abuse in the NE Region	March 24, 1998
EG on Scientometric Studies	March 25, 1998
Annual Review Meetings on Traditional Remedies for (i) Bronchial asthma and (ii) Diabetes mellitus	March 25, 1998
PRC on Tuberculosis, Leprosy, Viral Diseases and other Microbial Infections	April 15, 1998

Workshop/Seminar

A workshop on Cancer Registration under the National Cancer Registry Programme was organised at the Cancer Institute, Chennai (April 2-4, 1998).

A seminar on Situation Analysis of HIV and Drug Abuse in the NE Region was organised at the Regional Medical Research Centre, Dibrugarh (April 6-8, 1998).

Participation of ICMR Scientists in Scientific Events:

Dr. T. Adak, Asstt. Director, Malaria Research Centre (MRC), Delhi, participated in the Workshop on Validation of the Membrane Feeding Assay as a Tool for Development of Malaria Transmission - Blocking Vaccines at Bamako, Mali (March 21-28, 1998).

Dr. N.K. Ganguly, Director-General, ICMR, participated in the Indo-French Seminar on New Opportunities in Molecular Technologies at Bangalore (March 26-27, 1998).

Dr. N.K. Ganguly, D.G., ICMR, Dr. V.P. Sharma, Director, MRC, Delhi and Dr. S.K. Bhattacharya, Director, National Institute of Cholera and Enteric Diseases, Calcutta, participated in the III International Conference on Emerging Infectious Diseases in the Pacific Rim at Bali (March 28-30, 1998).

Dr. Aruna Srivastava, Asstt. Director, MRC, Delhi, participated in the GIS'98/RT'98 International Conference at Toronto (April 6-8, 1998).

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Dr. B.N. Saxena, Additional Director-General, ICMR and Chief, Division of Reproductive Health and Nutrition, retired on March 31, 1998.

COUNCIL'S TRAINING PROGRAMMES FOR 1998-99

Leprosy

At the Central JALMA Institute for Leprosy, Agra:

- Multidrug Therapy Orientation Course in Leprosy for Medical Officers (April 6-17, August 17-28, October 26-November 6; and December 7-18, 1998).

Virology

At the National Institute of Virology, Pune:

- Diploma in Medical Virology (July 1998-May 1999).

Reproductive Biology

At the Institute for Research in Reproduction, Mumbai:

- Workshop on Andrology for Clinicians (November 16-30, 1998)

Endocrinology

At the National Institute of Nutrition, Hyderabad:

- Annual Certificate Course on Endocrinological Techniques and their Applications (August 1-September 15, 1998).

Nutrition

At the National Institute of Nutrition, Hyderabad:

- M.Sc. in Applied Nutrition (June 1, 1998-February 28, 1999).
- Postgraduate Certificate Course in Nutrition (December 1, 1998-February 28, 1999).

Laboratory Animal Technology

At the National Centre for Laboratory Animal Science, National Institute of Nutrition, Hyderabad:

- Training Course for Laboratory Animal Technicians (June 16-July 31, 1998).

Occupational Health

At the National Institute of Occupational Health, Ahmedabad:

- Training Course on Environmental Hygiene Practice (November 16-20, 1998).

Biomedical Statistics

At the Institute for Research in Medical Statistics, Chennai:

- Basic Course in Statistics for Postgraduate Medical Students (July 1998).
- Basic Course in Statistics for Medical Officers (November 1998).

INDIAN COUNCIL OF MEDICAL RESEARCH

Grant-in-aid for organising Seminars/Symposia/Workshops

The Council provides partial financial assistance for organising Seminars/Symposia/Workshops. Applications for grant of financial assistance (complete in all respects in the prescribed proforma), will be considered only if furnished **at least four months before the date of commencement of the Seminar/Symposium/Workshop,** *etc.*

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