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Abstract A joint CDC/VBC team reviewed the Japanese encephalitis (JE) problem in Nepal and available in-country resources that could be used to address future JE outbreaks. Since 1978, when the first epidemic occurred, there has been a JE epidemic every other year, with increasing numbers of cases and deaths. In 1988, 1,371 cases of JE and 397 deaths were reported. For each case of human encephalitis there are an estimated 20 to 1,000 JE virus infections, suggesting that in 1988 there were between 27,000 and 1.4 million JE virus infections in Nepal. Infection with JE virus may result in a nonspecific viral syndrome and lost work days. JE vaccines are commercially available, but the expense and logistical difficulties associated with wide scale administration, coupled with difficulties in identifying target populations and questions about JE virus strain differences, preclude their use in Nepal at this time. Recommendations for short-term interventions that could be implemented with existing resources include water management in irrigated rice field mosquito breeding sites, use of bed nets and insecticidal coils, zooprophylaxis and alteration of animal husbandry practices. More long-term interventions, such as the use of biological control agents, will require comprehensive ecological data on the vector mosquitoes and their interaction with the biological control agents in Nepal.

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JAPANESE ENCEPHALITIS IN NEPAL:

RECOMMENDATIONS FOR SHORT AND LONG TERM CONTROL

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EXECUTIVE SUMMARY

The CDC/VBC consultant team reviewed the Japanese encephalitis (JE) problem in Nepal and available in-country resources that could be utilized to address future JE outbreaks. Japanese encephalitis has been monitored on a regular basis in Nepal since 1978 when the first epidemic occurred. However, the disease has probably been endemic in the terai for considerably longer. Epidemics have occurred regularly every other year with increasing numbers of cases and deaths. In 1988, 1,371 cases of JE were reported with 397 deaths. Encephalitis patients who survive may have permanent neurologic sequelae. For each case of human encephalitis, however, there are an estimated 20 to 1,000 JE virus infections, suggesting that in 1988 there were between 27,000 and 1,400,000 JE virus infections in Nepal. Infection with JE virus may result in a nonspecific viral syndrome, and may result in lost work days. Infection with JE virus thus has a significant health and economic impact on Nepal.

Although the use of insecticidal fogging during epidemics has political benefits, this intervention is ineffective in reducing JE virus transmission. Insecticides applied aerially for control of vector mosquitoes may be recommended as an emergency measure, but should be used only when active surveillance indicates an outbreak is imminent or is in the early stages. JE vaccines are commercially available, but the expense and logistic difficulties associated with wide scale administration, coupled with difficulties in identifying target populations and questions concerning JE virus strain differences, preclude their use in Nepal at this time.

Recommendations for short-term interventions that could be implemented with existing resources include use of water management in irrigated rice field mosquito breeding sites, use of personal protection measures such as bed nets and insecticidal coils, zooprophylaxis, and alteration of some animal husbandry practices. Implementation of these measures could be facilitated by using the district and village health educators to help farmers understand the problem. Longer-term interventions, such as the use of biological control agents, will require comprehensive ecological data on the vector mosquitoes and their interaction with the biological control agents in Nepal.

Other recommendations that can be initiated immediately with available resources include the following:

1. Improved surveillance for JE virus infection in humans and in reservoir and amplification hosts should be developed and implemented as early as possible. Concomitant entomological studies should also be undertaken that will incriminate the principal mosquito vectors of JE virus and determine their host preference and biting behavior. Studies on larval ecology should also address natural mortality factors which could be manipulated for enhanced control.

2. Two and possibly 3 persons should be trained in laboratory diagnosis of JE, field entomology and epidemiology as soon as possible. These persons could then be used to help develop and support the above surveillance and vector studies. If Ministry of Health, HMG, training priorities can be reasigned and support for travel and per diem for these individuals can be allocated, training could be provided without charge by the Division of Vector-Borne Viral Diseases (DVBVD), Centers for Disease Control (CDC), Ft. Collins, Colorado. Laboratory reagents and field equipment could be temporarily provided by DVBVD, CDC

3. Although the Central Health Laboratory (CHL) in Kathmandu is capable of doing basic JE virus serology using the IqM-capture ELISA, an additional individual (see No. 2 above) should be trained in arbovirology and assigned to the CHL staff to assume responsibility for support of the JE surveillance System.

Finally, multidonor support should be sought for development of a long-term national JE program, which would have 3 components: 1) an arbovirus laboratory dedicated to the JE problem; 2) a field epidemiology research program; and 3) community-based health education programs for prevention and control. This long-term program should be under the direction of the new Division of Malaria and other Vector-Borne Diseases. Adequate staffing for the laboratory and supportive field surveillance personnel will be essential in order for the laboratory and surveillance system to be completely effective.

I. INTRODUCTION

1.1 Historical Perspective

Among the mosquito-borne viruses that cause encephalitis in humans, Japanese encephalitis (JE) virus is the most important in terms of morbidity and mortality. It principally affects school-age children and causes high mortality rates and a high frequency of permanent neurological sequelae. JE virus is a flavivirus, a taxon originally encompassing group "B" arboviruses, and is closely related antigenically to Murray Valley encephalitis, St. Louis encephalitis, Rocio, West Nile, and Ilheus viruses. JE virus occurs throughout much of continental Asia and adjacent island groups.

Historically, the largest epidemics occurred in Japan and Korea. Major epidemics occurred in Japan until the late 1960s, but recently fewer than 100 cases per year have been recorded. The decline in incidence is attributed to changes in agricultural and pig-rearing practices, increased use of agricultural pesticides, urbanization, and an intensive JE vaccination program.

In recent years, the most extensive epidemics have occurred in the southwestern part of the JE virus range, especially in India, Burma, Nepal, Thailand and Sri Lanka. JE virus transmission may occur in tropical areas year around, but in higher latitudes outbreaks of JE are sharply defined and may occur only during one or two months of the year.

JE virus was first isolated in 1933 and subsequent research established that the virus has a complex transmission cycle involving vertebrates and vector mosquitoes. Basic studies on the ecology and epidemiology of JE virus in and around Tokyo by Scherer and Buescher (1959) incriminated Culex tritaeniorhynchus as the principal vector in that area. This mosquito breeds predominantly in rice fields and feeds on large domestic animals including swine, water buffalo and cattle, but also feeds on birds and man. In Japan Cx. tritaeniorhynchus overwinters as inseminated females, begins breeding in the spring and reaches a population maximum by late June.

The first sign of virus activity is its appearance in mosquitoes; not until 2 to 3 weeks later are infections detected in vertebrates, predominantly in black-crowned night herons, egrets and pigs. These serve as the main amplifying hosts.

Although the total vector population may soon begin to decrease, the proportion of the mosquito population that is infected increases, with a maximal density reached in late July.

The popularity of the pig as a meat source in the far east has undoubtedly been of importance in human epidemics. It is very attractive to the vector, circulates the virus in titers high enough to infect vector mosquitoes and has a rapid population turn-over due to slaughtering. In addition, pig breeding is scheduled to avoid economic losses of piglets from intra-uterine infection with the virus, and, as a consequence, the young are devoid of maternal antibody by the time epizootics develop.

Equine infections occur concomitantly with those of swine. The last event in the sequence is the occurrence of human infections. Man is not a preferred host for the vector and is not involved until a high density of infected mosquitoes is reached. It is believed that the virus is spread from rural to urban areas by viremic birds, which then initiate a secondary cycle.

Cx. tritaeniorhynchus is found both at ground level and at altitudes up to 50 feet so that it can amplify the infection among nesting birds. The rather prolonged breeding season of the black-crowned night heron enhances the supply of these susceptible hosts, which are important blood sources of the vector mosquitoes. Disappearance of the virus begins in late September and presumably is due to the disappearance of mosquitoes.

Major points that remain unexplained are the manner in which the virus survives during the winter and the source of virus for the mosquitoes first found infected early in the season. In the course of their studies, Scherer at al. obtained no evidence for the implication of wild rodents in the cycle.

1.1.1 Transmission of JE Virus

A number of mosquito species have been incriminated as vectors of JE virus in temperate, tropical and subtropical Asia. The principal vector throughout most of the range of JE, Cx. tritaeniorhynchus, is found in Nepal along with several other potential JE vectors, including Cx. fuscocephalus, Cx. bitaeniorhynchus, Cx. gelidus and the Cx. vishnui complex among others (Joshi et al. 1965, S. Pradhan unpublished data, Leake et

al., 1985 AFRIMS Rept.). Surveys by S. Pradhan (unpublished rept.) indicated that Cx. fuscocephalus was the predominant species found feeding on pigs (an amplification host for JE virus) followed in numbers by Cx. tritaeniorhynchus. However, light trap surveys conducted by Leake et al. (1985 AFRIMS Rept.) in the Eastern Region showed that Cx. gelidus was 10 times more abundant than either Cx. fuscocephalus or Cx. tritaeniorhynchus. Although JE virus has been isolated from Cx. tritaeniorhynchus in Nepal (Leake et al., personal comm.), its role as the primary vector of JE in the country has not been confirmed.

Intensive investigations in other areas such as Taiwan, Korea and Thailand have confirmed the zoonotic nature of JE virus, although the vector and vertebrate components may vary in different areas. For example, in Taiwan Culex vishnui (=annulus) may be a more important vector than Cx. tritaeniorhynchus (Detels et al. 1970, Mitchell and Chen 1973). In India, where JE virus has been isolated from a variety of mosquito species, the situation is less clear.

Little is known of host preference, biting behavior and habitat preference of JE virus vectors in Nepal. In several other countries, however, the bionomics of JE mosquito vectors has been studied in detail, especially in Japan, Taiwan and Thailand. Biting by females of most of the potential vectors usually takes place outdoors and after sunset. Although most of the known vectors prefer large mammals as hosts, birds have been implicated as important amplifying hosts of JE virus in several areas. The finding of viremic ducks in Nepal (D.D. Joshi, personal communication), indicates that species that are predominantly aviaphilic and susceptible to JE virus, such as Cx. bitaeniorhynchus, should also receive adequate attention.

Nearly all vectors of JE virus in Asia utilize rice fields and other sites as larval habitats. Certain species, such as Cx. tritaeniorhynchus, use rice fields as the main breeding site. Rice is extensively cultivated throughout the endemic zone of JE in Nepal. Rice cultivation is fast replacing formerly forested areas in the terai and extending the habitat of JE vectors considerably. Larval surveys by Pradhan (unpublished rept.) and Leake et al. (1985 AFRIMS Rept.) have demonstrated the presence of several potential JE vectors in rice fields as well as other breeding sites. Considering their findings and those of researchers in other countries (reviewed by Mitchell 1977), along with the extensive cultivation of rice in Nepal, it is highly probable that this habitat is the predominant source of larval breeding.

It is apparent that a variety of entomological aspects of JE transmission must be elucidated before anti-vector efforts can be effectively implemented. These are outlined in detail in the recommendations.

1.2 Situation in Nepal

In Nepal JE has been recognized as a significant public health problem only during the past decade (Joshi 1986, 1987). The disease has been reported from each district in the terai and in adjacent districts of India (Joshi 1987, Bhardwaj et al. 1981). At the beginning of 1980, an epidemiologic study was started in all terai districts and records of morbidity and mortality were collected and analyzed for all age and sex groups for the period 1978-1984.

During the period 1978-1984 a total of 2,508 cases of suspected JE were admitted in various hospitals and 886 patients died (35.3% mortality rate). Among 1,505 human and 299 mammal and bird sera tested for haemaglutination-inhibition (HI) antibody to JE, 33.4% of the human sera and 33.8% of swine sera had antibody titers of 1:10 or greater (Joshi 1986). Most human cases occurred among individuals older than 15 years of age (36.7%) and among school children aged 5-14 (32.3%). Only 11.3% of the cases were under 5 years of age; the age of 19.7% of the cases was not specified. The mortality rate among children younger than 14 years of age was 36.9% and 45.5% among individuals 15 or older (Joshi 1986).

Further epidemics of JE occurred in 1985-86 and 1988 (Joshi 1987, MOH unpubl. data). Ten districts in the terai were affected in 1985 and 13 districts in 1986, whereas during 1988 all Terai districts were affected and cases were reported from other districts as well.

During the 1985 and 1986 epidemics, collaborative studies between the MOA, MOH, U.S. Armed Forces Research Institute of Medical Sciences (AFRIMS) Bangkok, a British Army Medical team, and the London School of Hygiene and Tropical Medicine confirmed that JE virus was the etiologic agent (Joshi 1987, C.J. Leake, personal communication) in the Morang District of the Koshi Zone of East Nepal. JE virus isolates were obtained from human brain and cerebrospinal fluid (CSF) samples, a sentinel pig and a pool of 100 *Cx. tritaeniorhynchus* females collected 10 August 1985 before the apparent peak of human disease in October. Mosquitoes also yielded isolates of the alphaviruses Sindbis (2) and Getah (10) and 7 untyped virus isolates. Although 219,427 mosquitoes were collected, only 171 pools comprising an undisclosed number of specimens were tested for virus.

Only a portion of the mosquito collections were identified to species; however, at most sites Cx gelidus was up to 10 times more prevalent in light trap collections than the other most abundant mosquitoes Cx fuscocephalus and Cx tritaeniorhynchus.

In 1986 JE virus IgM and IgG antibody capture ELISA tests indicated that 119 of 124 suspect clinical cases were infected with JE virus (Leake et al. personal communication). The overall fatality rate in confirmed JE cases was 21.6% with a male to female ratio of 1.5:1 among fatal cases. Mortality rates were higher in males over 15 years of age (39.5%) as compared to younger males (11.6%), but no such difference in female fatality rates was noted (Leake, per. comm.).

The seasonal incidence of clinical JE in Nepal for the period 1978-80 was summarized by Joshi (1986). Apparently, low level transmission of JE virus occurs throughout the year in the terai. However, during 1978-80 and 1983-86, discrete peaks occurred during September and October at the end of the monsoon (Joshi 1986, 1987).

2. Current Situation

2.1 Epidemiology

The incidence of JE in Nepal has increased progressively since the first documented epidemics occurred in 1978. Epidemics have occurred at approximately two - year intervals (see section 1.2) and it is anticipated that this cycle will continue indefinitely. Since 1978, each successive epidemic has generally been larger (Figure 1). In 1988, 1,371 cases were reported. Districts with the highest number of cases included Morang (East region), Rupendahi and Kapilbastu (West region) and Bankey and Bardia (Mid-west region), (See Annex 2.)

The overall case fatality rate in Nepal has been generally high, ranging from 14.8% in 1983 to 46.3% in 1982 (Figure 1). On the average, case fatality rates can be expected to be about 30%, depending on the district and the medical services available. It should be noted, however, that case fatality rates are generally underestimated because a significant number of patients (190 in 1988) leave the hospital against medical advise (Lama) when death is imminent. Sex and age distribution of 1988 cases are presented in Annex 3.

JE is currently widespread in the terai of Nepal (Figure 2). The first epidemics occurred in the eastern (Koshi Zone) and central (Lumbini Zone) areas of the country, but cases of JE have

subsequently been reported in all districts of the terai. Most likely, JE virus is endemic throughout the terai and possibly in parts of the inner terai as well. In 1988, cases of JE were reported from districts in all five regions. The highest incidence, however was in the east, west and mid-west regions.

Currently available data suggest that the JE problem in Nepal will become progressively worse if effective intervention strategies are not implemented. Unfortunately, before effective prevention and control can be developed, more information must be obtained on the epidemiology of JE virus in Nepal.

We can only speculate as to why epidemics apparently began suddenly in 1978. (There is some evidence that JE epidemics occurred earlier in 1960 and 1968, see Joshi, 1986.) It is possible that JE virus has always been present in Nepal, but severe disease incidence in humans was never high enough to be recognized as a clinical entity until major demographic changes occurred in the 1960s and 1970s, when large numbers of people moved into the terai from the mountains and rice cultivation expanded dramatically.

Very little is known about the basic JE virus maintenance and amplification cycles in Nepal. While there may be similarities to other endemic areas of Asia, some evidence suggests that there may also be differences. The primary mosquito vectors of JE virus in Nepal are not known. Although most mosquito species that serve as vectors in other endemic areas of Asia are present in Nepal, JE virus has been isolated from only one species (Cx. tritaeniorhynchus), despite the many thousands of mosquitoes that have been collected and tested. The virus has been isolated from a sentinel pig (Leake, per. comm.). JE virus has not been isolated from other potential mosquito vectors, nor from other potential vertebrate reservoir hosts.

Information is equally scarce about the amplification cycle that is responsible for increasing the risk of human exposure to JE virus infection. In one study that used sentinel pigs, none of the sentinels seroconverted in the first year (1984). During a period of higher transmission in the second year of study (1985), only 2 out of 12 seroconverted to JE virus infection (Leake, et al., AFRIMS report). In similar studies in other endemic countries, the seroconversion rate in pigs has generally been much higher. Also of interest is that JE cases have been reported in areas of Nepal where there are no domestic pigs, suggesting that other vertebrate hosts, such as ducks, may be involved in peridomestic amplification of the virus. Another unknown is the role that wild pig populations in these areas play in basic maintenance or amplification cycles.

More information is also required about the etiology of viral encephalitis cases in Nepal. While it has been well documented that JE virus is responsible for many of the cases in the eastern region, there has been no systematic study of the etiology of viral encephalitis cases in other parts of Nepal.

2.2 Resources Available

2.2.1 Laboratory

The Nepal Ministry of Health has one Central Health Laboratory (CHL) located in Kathmandu. This laboratory is responsible for diagnosis of all parasitic and infectious diseases except malaria. The CHL supervises a network of clinical laboratories located in zonal hospitals throughout the country. However, the latter are only capable of carrying out relatively simple clinical laboratory tests.

The CHL in Kathmandu is well equipped and has a trained microbiologist/virologist who is responsible for diagnostic virology. She has been trained in cell culture and a variety of virologic and serologic techniques that are required for JE virus diagnosis. Currently, only the I_gM-capture ELISA is being done at CHL, although they also have the capability to do the hemagglutination-inhibition (HI) test.

The CHL has all necessary equipment to carry out virologic and serologic studies on JE virus. However, the laboratory is not capable of producing the reagents required for arbovirus diagnosis. These will have to be supplied by a reference laboratory.

The CHL is short of staff and apparently has had difficulty maintaining adequate support for JE field studies in the past. The microbiologist/virologist who does the JE diagnostic work is also responsible for other virus diseases such as HIV, hepatitis and measles.

Space at the CHL is also at a premium. The many functions of the laboratory have made it necessary to carry out diagnostic work for several etiologic agents in the same laboratory. Even so, limited space for the JE diagnostic work can be made available on a temporary basis (Dr. V. L. Gurubacharya, personal communication).

2.2.2 Human resources.

There are few well trained scientists available in Nepal to deal with the JE problem. Most of the scientists who are adequately trained are already overloaded with work in other areas such as malaria and other infectious diseases. The one Nepalese

epidemiologist who is a world expert on JE has been transferred back to the Ministry of Agriculture, where he worked prior to his JE studies.

Currently there are no trained epidemiologists nor entomologists working on the JE problem in Nepal. In the laboratory there is only a single microbiologist/virologist who is capable of doing diagnostic virology and serology for JE. There is a desperate need, therefore, to identify and train epidemiologists, entomologists and virologists to develop programs on surveillance, prevention and control of JE in Nepal.

3. Programs that can be Implemented Immediately

3.1 Intervention Strategies

3.1.1 Insecticides

3.1.1.1 Adulticides: The mosquito vectors of JE normally feed outdoors. Therefore, indoor residual insecticide spraying, as carried out for malaria control, is not effective for prevention and control of JE.

Adult mosquito control using ultra low volume and thermal fog application of insecticides, however, has been widely recommended for control of a variety of human disease vectors, including those that transmit JE virus. Insecticide fumigation for JE vectors has been applied using both ground equipment and airplanes. Reports on efficacy have been variable, some showing temporary reduction in adult mosquito densities. None, however, have convincingly documented that fumigation for adult mosquitoes has had a significant impact on disease transmission. Moreover, insecticides and their application are very expensive.

It is not recommended, therefore, that adulticides be used for prevention and control of JE mosquito vectors in Nepal. It is recognized, however, that limited use of adulticides may be necessary to demonstrate to the people that the government is interested in their problem and trying to do something about it.

3.1.1.2 Larvicides: Insecticides applied to larval habitats can be very effective in temporarily reducing mosquito populations. However, they are very expensive and after continued use, the mosquitoes may rapidly develop insecticide resistance. Another consideration in using larvicides on the mosquito vectors of JE is that they normally breed in rice fields. This presents a major problem in delivery of the insecticides. In such a situation larvicides can be applied using back pack sprayers or by airplane. The former is very labor intensive and the latter very expensive.

The use of insecticides for routine control of JE mosquito vectors in Nepal, therefore, is not recommended. In the event of a large epidemic, some authorities might recommend aerial fumigation. It should be noted, however, that surveillance is generally very insensitive, and in most epidemics, transmission has usually peaked before an aerial spray program can be implemented. It thus has little or no impact on disease transmission. Aerial spray can only be justified if surveillance is improved to the point where epidemic activity can be accurately predicted well in advance of peak transmission.

3.1.2 Non-chemical Intervention

A number of options are available for control of JE vectors without the use of insecticides in both the near- and long-term. These include environmental management, zooprophylaxis, animal husbandry, personal protection and biological control. Each is discussed in more detail below.

3.1.2.1 Environmental Management: One of the oldest methods of mosquito control involves the reduction of habitat suitability through water management. Areas that are most amenable to this strategy are those in which irrigation is the main source of water. Intermittent irrigation, or providing water long enough to sustain optimal growth of rice plants without allowing water to stand long enough to permit mosquito breeding, is the most common form of environmental management in rice fields. Such practices have been successfully implemented in China and Japan. Intermittent irrigation results not only in reduction of mosquito densities, but also in reduced water consumption (Figures 3 and 4). Other variations include use of drought resistant cultivars and early maturing varieties that require considerably less water and hence reduce the availability of larval habitats.

Mogi (1984) suggests periodic flushing of rice fields to remove mosquito larvae, but points out that the method depends on an excess of irrigation water, irrigation systems designed to induce water currents over the whole area that will carry larvae to sites unfavorable for their development and procedures to kill large concentrations of larvae if necessary. This method may have less impact on predator populations than the complete drainage of fields used in the intermittent irrigation method.

Another method suggested by Mogi (1984) is to vary planting schedules to avoid coinciding with the JE transmission season. This strategy would only be useful where rainfall or irrigation would permit a shift in planting time.

3.1.2.2 Zooprophylaxis: The mosquito vectors of JE have very specific blood feeding and resting behavior. Most prefer to feed outdoors in the early evening for a few hours after sunset. Moreover, most prefer to feed on large bovine species such as cattle and buffalo. While the resting behavior of these species is not fully understood, it is known that most prefer to rest outdoors and that most blood seeking females come from the rice fields. This information can be used to reduce the probability that a female mosquito infected with JE virus will feed on a human, and thus reduce the incidence of JE virus infection in humans.

The rationale of zooprophylaxis as an intervention strategy is that if a number of preferred hosts, i.e. cattle or buffalo, are placed between the rice field where most resting mosquitoes occur and living compounds where most evening human activity occurs, the mosquitoes emerging from the paddy will choose to blood feed on the preferred barrier animals rather than proceed to the human compounds to feed on man or other animals. This animal barrier can be easily implemented by the farmer at little or no expense. It may not be completely effective alone but should contribute to reducing the probability of human infection, especially when used in conjunction with other methods outlined in section 3.

3.1.2.3 Animal Husbandry: Swine and other domestic animals such as ducks are excellent hosts for JE virus and infected animals may circulate virus in their peripheral blood. Vector mosquitoes feeding on these animals readily become infected with JE virus. As a result, they act as amplification hosts, infecting more mosquitoes in the immediate vicinity of human compounds, thus increasing the chances of human infection. It follows, therefore, that preventing infection of pigs and ducks should decrease human infection as well.

Certain animal husbandry practices may help reduce JE virus infection of pigs. Since the mosquito vectors prefer to feed outdoors, if pigs can be maintained indoors or if they can be moved indoors during the early evening hours, reduced mosquito feeding activity should result, thus lowering infection rates in this species and in humans.

3.1.2.4 Personal Protection: Bed nets have been used for hundreds of years to protect humans from mosquito bites. More recently impregnation of bed nets with fast acting insecticides, such as the pyrethroids permethrin or deltamethrin, has extended the effectiveness of nets. In addition to the insecticidal action of the pyrethroids, the impregnated nets are actually repellent to mosquitoes. Thus, torn nets or those with holes are more effective after impregnation than untreated nets. Although JE vectors generally feed outdoors, nets can still

provide protection to individuals sleeping outdoors during the transmission season. Use of bed nets also affords a degree of protection against malaria transmission.

Bed nets tend to be most effective in populations that have demonstrated a tendency to use such measures of their own volition. Community education about the benefits of bed nets, especially those that have been impregnated with insecticide, will further enhance or encourage the use of this intervention strategy. Commercially available insecticidal coils are another form of personal protection that reduce man-vector contact, mostly indoors.

3.1.2.5 Biological Control: Various means of biological control have been successfully used against riceland mosquitoes, including larvivorous fish and microbial control agents. Optimal results will be obtained with biological control agents if the ecology of the target species is thoroughly understood prior to implementation.

When properly managed and when used in sufficient numbers, some species of fish have effectively reduced mosquito larvae in rice fields in China and Afghanistan. The mixture of pisciculture and rice cultivation in China has resulted in both biological control of mosquitoes and concomitant production of edible fish.

Use of fish in Nepal, however, should follow ecological studies on the larvae of JE vectors and small scale pilot studies on the feasibility and efficiency of using fish for larval control. Some possible disadvantages to the use of fish are: they must be used in mass releases in rice fields since this habitat does not permit long term establishment; they may reduce native populations of predatory insects; and exotic species may replace native species of fish.

The most successful biological intervention to date in rice fields has been the use of microbial control agents (*Bacillus thuringiensis var. israelensis* [Bti], *B. sphaericus* and *Lagenidium giganteum*). Of the bacteria, *Bti* is commercially produced and used routinely for control of riceland mosquitoes in Arkansas, parts of Louisiana and California. Effective rates range from 3-5 oz/acre (220-365 ml/ha) when applied aerially with ULV spray equipment.

In order to be effective as an intervention for JE control, larviciding with *Bti* must precede epidemics. If JE outbreaks can be predicted with accuracy, larviciding in rice fields in the immediate vicinity of populations at risk could play a significant role in reducing the extent of epidemics.

Due to the poissonian distribution of the larvae of Culex vectors in rice fields, and their propensity for aggregating near the edge of rice fields, selective spraying in these areas could reduce the amount of Bti and labor necessary for control.

One of the greatest advantages of Bti for mosquito control is its selectivity. Nontarget organisms, including natural insect predators of mosquito larvae are unharmed and hence will be present when the habitat is reinvaded by mosquitoes. Unfortunately, there are several disadvantages to using Bti. Because the active moiety is a large polypeptide, it is rapidly denatured in the environment, drastically curtailing larvicidal activity. The cost for vector control using Bti in developing countries is somewhat prohibitive and only feasible in the presence of a sound JE surveillance system.

The other two most promising candidate microbial control agents, B. sphaericus and L. giganteum demonstrate exceptional potential for control of riceland mosquito larvae. In many cases these agents are more active and provide greater residual control than Bti. They are currently under experimental use only, but are being strongly considered for commercial production. The fungus, L. giganteum, offers the additional benefit of recycling within the target population and reducing larval populations over an extended period. All of these microbial agents can be applied using conventional insecticide application equipment.

3.1.3 Community Education

Unfortunately, the general public in most countries of the world has been taught to rely on government agencies to solve their health problems. It will be noted in Section 3.1.2 above, however, that most of the non-chemical intervention strategies listed require the help of the farmers who live in the JE endemic areas.

Considerable public health education will be required, therefore, to change public perception about the disease problem, its origin, and how to implement effective prevention and control.

Ministry of Health scientists should organize training programs on JE for regional and district health educators. Educational materials should be developed using simple language and art that explain the disease and how the public can help prevent infection. These materials should be developed with the input of regional and district health educators who understand local customs and culture. Programs should also be developed to help local farmers modify and implement intervention strategies that are appropriate to their specific areas and conditions.

Health education is a long-term process that requires constant evaluation and revision by the local health educators. The latter should be encouraged to work closely with their community and to be innovative in developing and trying new approaches to communicating with the public. These programs will require some central financial support, but health educators also should be encouraged to seek support from local business and civic organizations. Involvement of these groups, which are frequently absolved of any responsibility for community health, will ensure a truly community-based prevention and control program.

3.1.4 Vaccination

3.1.4.1 Humans: Crude, formalinized mouse-brain vaccines were tested in Japan in the late 1930s and licensed in 1954. Purification with protamine sulfate became a routine commercial procedure in 1962. A large double-blind placebo-controlled trial of protamine sulfate purified vaccine prepared from the Nakayama virus strain was conducted in Taiwan in 1965 and showed an efficacy rate of 81% following two doses (Hsu at al. 1971). The efficacy of more highly purified products were compared in an extensive trial in Thailand by Hoke et al. (1988). They used two doses of monovalent (Nakayama strain) or bivalent (Nakayama plus Beijing strains). They reported a 91% efficacy in children who received 2 doses, compared to placebo, and no difference between the monovalent and bivalent vaccines.

The monovalent Biken vaccine (Nakayama Strain) costs about $2.30 per dose. Less expensive inactivated and live, attenuated vaccines produced in tissue culture have been used successfully in China (Huang 1982). The best of the live vaccines is designated SA-14-14-2.

Studies in Nepal and the United States using the Biken vaccine indicate that a 3-dose regimen may be required to produce a protective antibody response in more than 90% of recipients (Henderson, unpubl. report, Poland et al. in Press). Pavri (1984) reported that among 111 persons given 2 doses of the Biken vaccine in India, 57% did not develop neutralizing antibodies. Antigenic variants of JE virus in different parts of its range (Kitano et al. 1986, Bannerjee 1986) raise the question of whether monovalent vaccines from one area are protective throughout the range of the virus.

Aside from the question of efficacy, the use of JE vaccine poses the usual problems associated with epidemic zoonotic diseases that occur in an unpredictable fashion. Assuming that financial

resources for the vaccine and its administration can be found, it may be difficult to identify the target population at greatest risk. Also, a vaccination program would have to be continued indefinitely since non-immunes are constantly entering the population and since vaccination would not affect the level of JE virus transmission in its basic maintenance cycle.

3.1.4.2 Horses: Other than man, horses are the only animals in which JE virus causes a fatal encephalitis. Extensive epizootics of equine encephalitis occurred in Japan prior to 1948 when a vaccination program was implemented using an inactivated vaccine. Horses are attractive hosts for *Cx.tritaeniorhynchus* and horses develop JE viremia; therefore, in endemic areas where equines are abundant, vaccination against JE virus might reduce the pool of potential amplifying hosts.

3.1.4.3 Pigs: The role of pigs as amplifier hosts of JE virus is well established in east Asia and vaccination of swine has been suggested as a control measure for reducing the number of mosquitoes that become infected when they feed upon swine. An attenuated, live-virus vaccine is available for use in swine and has been used to prevent stillbirth from JE infection *in utero* by vaccinating gilts. However, the impact of such vaccination on JE virus transmission in discrete foci remains to be elucidated. Also, the presence of maternal antibody in newborn piglets for approximately 4 months after birth makes the timing of vaccination difficult.

3.2 Epidemiology

There is a general lack of knowledge about JE disease in Nepal (See section 2). A better understanding of distribution, etiology, mosquito vectors and vertebrate hosts would allow the development and implementation of more effective methods of prevention and control. Several areas where critical programs can be implemented immediately are outlined below:

3.2.1 Surveillance

A laboratory based surveillance system is essential to determine distribution, incidence and etiology of viral encephalitis in Nepal. Before improved case detection can be achieved, a specific case definition must be agreed upon and used by all physicians. Seminars should be arranged to educate the medical community in endemic areas about clinical diagnosis and treatment of JE disease using a standard protocol. A weekly case reporting system for suspected viral encephalitis cases should be implemented and enforced at the district level, using a standardized clinical data collection form. Data analysis can be facilitated by using the new computer system being set up for the National Malaria Eradication Organization (NMEO).

3.2.2 Etiology

It is important to determine the etiology of viral encephalitis in Nepal. This can be accomplished under the current system of hospital laboratories that is supervised by the Central Health Laboratory (CHL) in Kathmandu. As part of the above surveillance system, paired serum samples and cerebospinal fluid (CSF) should be collected from suspected viral encephalitis cases. In addition, brain biopsies, heart blood and CSF should be taken from all cases of encephalitis with a fatal outcome. (Brain biopsies can be easily obtained by inserting a Vim Silverman needle into the brain through the nose).

Many people leave the hospital against medical advice (Lama) when death is imminent (See Annex 3). This practice should be discouraged because it may prevent determination of the etiology of these cases. Moreover, fatality rates are underestimated because these patients are not included in reports of fatal cases.

A transport system for laboratory specimens is already in place at the CHL in Kathmandu. Samples are routinely shipped by bus to CHL from outlying district hospitals in styrofoam boxes on icepacks. This system can be used to send the above samples to Kathmandu on a weekly basis.

As a service to the Ministry of Health, the Division of Vector-borne Viral Disease (DVBVD) Centers for Disease Control (CDC) laboratory in Fort Collins, Colorado, USA, can provide virologic diagnosis on these specimens on a temporary basis, if desired. Arrangements can be made to ship specimens to DVBVD each month or less frequently, depending on the number of specimens collected. Since the CHL does not do IgG serology, serum and CSF samples can also be sent to DVBVD CDC for some serologic diagnosis.

3.2.3 Training

Ultimately, all virologic and serologic diagnosis should be done in Nepal. To accomplish this, however, another person must be trained and assigned to CHL to work exclusively on JE virus. Dr. Gurubacharya, superintendent of CHL, indicated that space and basic support could be made available for such a person, at least temporarily. Provided the MOH can find funds to support this training, a microbiologist virologist could be sent to DVBVD, CDC for a 3 month training course on virologic and serologic diagnosis of JE virus infection. This training would include the latest techniques for measuring IgM and IgG antibodies, tissue culture and virus isolation and identification

of JE virus from autopsy tissue, CSF, serum and mosquito pools. In addition to training, DVBVD, CDC can also temporarily supply CHL with the reagents needed for virologic and serologic diagnosis of JE virus.

If funds can be found, the DVBVD, CDC could also provide training for a vector ecologist who is needed to implement studies to identify primary mosquito vectors and vertebrate hosts. Training for this individual would include mosquito surveillance and collection techniques, mosquito taxonomy, mosquito age grading techniques, processing mosquitoes for virus isolation, blood meal identification and methods for collecting and bleeding avian and other vertebrate species. It is anticipated that this training would also take about 3 months.

If MOH training priorities can be changed and funds identified without delay, both of the above individuals could be trained before the next transmission season arrives in September, 1989. The trainees could arrive in DVBVD, CDC by mid May, and return to Nepal by late August to set up the laboratory and field studies needed to obtain the information outlined in Section 2.

Initiation of both laboratory and field programs in Nepal would be facilitated if a laboratorian and a vector biologist from DVBVD, CDC, returned to Nepal with the trainees. The laboratory in Kathmandu could then be made operational while initial field studies on mosquito vectors and vertebrate hosts were being implemented. Participation in these programs by DVBVD, CDC personnel, however, would be contingent upon availability of travel funds.

3.2.4 Field Studies

3.2.4.1 Incrimination of Vector Species: Upon the return to Nepal of the individual trained in vector ecology, a field program should be implemented at field sites in the epidemic region prior to the peak JE virus transmission season in 1989. The major objective of this study would be to collect large numbers of female *Culex* mosquitoes using a variety of trapping methods, identify these to species, pool each species in lots <100, and return them to the central laboratory for virus isolation tests. This work could be done at CHL in Kathmandu. After the important vector species during the epidemic season have been identified, detailed studies on the bionomics of the vector(s) can be undertaken.

3.2.4.2 Incrimination of Vertebrate Hosts: Studies will be initiated at the same site(s) selected for the mosquito collections to determine the role of mammals and birds in the amplification of JE virus during the epidemic season. In view of

previous results from Nepal and elsewhere, special attention should be given to swine, equines, ducks, herons and egrets. This will be accomplished by collecting blood samples, separating sera, and sending these to the CHL in Kathmandu for serologic or virus isolation tests as appropriate.

4. Long-Range Requirements for Sustained Management of Japanese Encephalitis in Nepal

4.1 Introduction

As noted in Section 2, JE is an emerging disease problem in Nepal. Available data on epidemic trends indicate that both disease incidence and distribution will continue to increase if intervention strategies are not implemented. Effective prevention and control, however, cannot be initiated without more basic information about the disease in Nepal.

Many of the short-term intervention programs outlined in section 3 will need to be expanded and sustained in the proposed long-term program. The need for this approach is dictated by the paucity of information about etiology of encephalitis cases, primary and secondary mosquito vectors and their bionomics, primary and secondary vertebrate hosts, human behavior and cultural practices that might influence transmission, distribution of JE virus in Nepal and the clinical expression of JE virus infection in humans.

The economic impact of JE virus infection in Nepal is unknown. In most JE endemic areas of the world, it is estimated that there are between 20 and 1,000 JE virus infections in humans for every case of serve disease. Thus, it can be estimated that there are between 20,000 and 1,000,000 JE virus infections in Nepal during an average year when 1,000 cases of encephalitis are reported. It should be noted that while these patients may not have severe neurologic disorders, many may have a viral syndrome that lasts from 3 to 7 days. This obviously has a significant impact on productivity in those areas of the country that are affected. If this significant morbidity is considered along with an average 1,000 severe encephalitis cases per year (average of last 4 years) and an average of about 300 deaths (not including those patients who leave the hospital when death is imminent) each year due to JE, this disease is extracting a major economic toll on Nepal.

In addition, JE virus infection in the swine population may be important economically because it causes stillbirths in pregnant sows. Data on the impact of JE on the swine industry in Nepal are not available.

It is clear from the above discussion that JE is an important public health and economic problem in Nepal. Unfortunately, there are no immediate measures that can be implemented to reverse the trend of increasing incidence of disease. Only through establishment of the infrastructure necessary to study this disease in Nepal can long-term, effective intervention strategies be developed.

4.2 Building an Infrastructure to Support JE Studies

Nepal MOH officials indicated that the NMEO and the Division of Epidemiology are being reorganized as part of the overall decentralization program in the MOH. It is not entirely clear at this time just how this will effect the proposed program, but all indications are that it will facilitate development of a national JE program. Thus the proposal to move JE and other arbovirus diseases to a new division called Malaria and other Vector-Borne Diseases should allow the use of the present NMEO infrastructure in developing the JE field program.

Another area where decentralization can be utilized to facilitate development of a national JE program is in health education. The proposed plan to establish a health education office in each region of Nepal and to have an assistant health educator in each district could be used to develop and implement community-based health education for prevention and control of JE.

Finally, the current NMEO infrastructure, in collaboration with the CHL hospital laboratory infrastructure, can be used to facilitate long-term surveillance for JE virus infection.

4.2.1 Arbovirus Laboratory

Any long-term national JE program must have its own laboratory system. While initial studies can rely on current space and equipment available at CHL, a new arbovirus section of that laboratory will ultimately have to be built in order to sustain a long-term JE program. Alternatively, a new laboratory could be established in the Division of Malaria and other Vector-Borne Diseases. The latter option would be preferable because it would allow the diagnostic and surveillance activities to be under the same administrative control as the field research program.

In either case, development of an arbovirus laboratory will require training new personnel, finding adequate space and purchasing equipment and supplies.

4.2.1.1 Laboratory personnel. An arbovirus laboratory must have minimal staffing as follows:

Staff Members	Training	Number
Chief of Laboratory	Microbiologist/ Virologist	1
Senior Laboratory Technician	Virology	1
Senior Laboratory Technician	Serology	1
Laboratory Technician	Tissue Culture	1
Laboratory Technician	Computer	1
Laboratory Assistant	General	2
Laboratory Aid	General	3

Training for the above individuals can be provided in both the U.S. and Nepal. The laboratory chief and three of the laboratory technicians should be trained at the DVBVD, CDC laboratory in Fort Collins, Colorado. Training periods would be 3 to 4 months, depending on the qualifications and background of the individuals. The rest of the staff can be trained in Nepal as the laboratory is being developed.

4.2.1.2 Laboratory space. Space requirements will not be great. In general, a moderately sized room will be needed for each of the following functions: general virology, serology, specimen processing, tissue culture and washroom. Smaller rooms will be required for immunofluorescence and for working with mosquitoes. In addition, a room in a separate building will be required for rearing mosquitoes.

4.2.1.3 Equipment and supplies. Basic equipment and supplies required to make the laboratory operational are outlined in Annex 4. It is estimated that the initial start-up cost of this laboratory would be approximately $150,000, U.S.

One equipment item that is not strictly laboratory is a computer. An AT or IBM compatible unit is required to handle incoming specimens. A computer-based sample logging program is available from the Dengue Branch, DVBVD, CDC, that uses data base III software. This system can be easily adapted to the JE laboratory program, thus providing appropriate health officials with easy access to information.

4.2.2 Surveillance

Long-term prevention and control of JE in Nepal will ultimately depend upon an effective surveillance system that reaches to the district level. Basic needs of such a system are outlined in Section 3.4. That outline can be expanded and improved as the arbovirus laboratory is being developed.

The JE surveillance system should ultimately be computer based, using the NMEO computers that are currently being installed and the laboratory computer. The CDC logging program is written to identify geographic regions by patient identification members. The current geopolitical divisions used by the Nepal MOH can easily be adapted to that program.

4.3 Field Studies

4.3.1 Distribution of JE in Nepal

Long-term seroepidemiologic studies will be required to define in detail the populations at greatest risk of contracting JE. Such information will be essential for planning intervention strategies based on vaccination campaigns or vector control programs. Active case finding and serologic confirmation of cases will be essential to obtain reliable data.

4.3.2 Etiology of viral encephalitis cases

Clinically, JE may be confused with other diseases caused by a variety of pathogens. Therefore, definitive diagnosis must be based on serologic or virus isolation results. Physicians in the zonal hospitals must be trained in clinical diagnosis of JE and alerted to the need for obtaining appropriate serum and CSF samples from suspect cases as soon as possible after the onset of illness and canvalescent serum 2 to 6 weeks later. Brain samples, heart blood and CSF should be obtained from cases with fatal outcome for specific diagnosis using virus isolation and fluorescent antibody tests.

Several virus isolates should be obtained from different geographic regions within the terai, and from different sources, e.g., human cases, mosquitoes, pigs and birds, for antigenic characterization by an arbovirus reference laboratory. This information will be needed to determine whether JE viral strains differ in Nepal and whether they are distinctive from isolates from other countries. Such information will be vital to selecting commercially available JE vaccines or candidate viral strains for vaccine production.

4.3.3 JE virus amplification cycles

Studies in different ecologic settings will be required to identify the principal vertebrate hosts involved in the amplification of JE virus throughout its range in Nepal. The relative importance of various mammalian and avian species can be expected to vary from one area to another, and such information is vital for implementing effective prevention and control. Methodologies will consist mainly of those used in the short-term study and will involve censuses and serosurveys.

Studies on potential vector species should be undertaken at the same time and at the same sites. Following incrimination of the principal vector(s) as outlined in Section 3.2.4, intensive studies on their bionomics should be initiated.

4.3.3.1 Vector Bionomics: The seasonal population dynamics of the vector mosquitoes should be described by using a variety of sampling methods for adult and larval mosquitoes. Dissection of samples of adult females to determine physiological age will provide useful information on the age stratification of populations and allow inferences to be made about the number of blood meals a female is likely to take during her lifetime.

The frequency with which mosquitoes are observed biting humans indoors and outdoors with respect to time of day must be determined. Such information will clarify the most likely time and place of virus transmission and provide insights on potential intervention strategies.

Collection of blooded mosquitoes should be made throughout the year to determine seasonal feeding patterns. Mosquito blood meals should be identified by precipitin test or other means, and special attention should be given to detecting feeding on humans, bovines, pigs and birds.

The potential for infected mosquitoes to infiltrate human population areas should be investigated using mark-release-recapture techniques to determine flight range and resting habits.

4.3.4 JE virus maintenance mechanisms

JE incidence data suggest that low-level transmission of JE virus may occur year round in the terai (Joshi 1986). However, this assumption is based on clinical diagnoses and not serologic confirmation. A first priority should be to determine whether year round JE virus transmission does occur in the terai. If so, no further search for ancillary maintenance mechanisms seems warranted. In addition to seeking serologic confirmation of clinical cases of JE, the use of sentinel animals, such as swine and ducks, may provide a ready answer to this question.

If year round transmission of JE virus cannot be documented, other studies designed to elucidate maintenance mechanisms should be implemented. These should include 1) vertical transmission of JE virus by infected female mosquitoes, 2) chronically infected vertebrate hosts, and 3) annual reintroduction of JE virus by migrating birds or other means. JE virus has been isolated once from ticks in the USSR; therefore, the possibility of a tick reservoir should also be considered.

4.3.5 Human behavior and practices

Village health workers may be able to define behavioral and cultural activities that are important in increasing or decreasing the risk of rural inhabitants coming into contact with vector mosquitoes. If so, education campaigns could be mounted to obtain community support for implementing changes that would reduce that risk.

4.3.6 Human vaccine trial

If the seroepidemiologic studies described in Section 4.3.1 are successfully completed, it may be practical to administer vaccine to discrete populations at highest risk.

4.3.7 Vaccination of Animals

See section 3.1.4

4.3.8 Other arboviruses

Two alphaviruses, Sindbis and Getah, have been isolated from mosquitoes in Nepal, as well as 7 untyped virus isolates (Leake, per. comm.). Dengue also has been reported from Nepal; however, serological confirmation and virus isolation are lacking. Among these viruses, Sindbis and dengue viruses are known to cause clinical disease in man. The clinical syndrome may be similar; therefore, specific diagnosis must be based on appropriate serologic tests. The seroepidemiologic studies (Section 4.3.1) and those designed to elucidate the etiology of viral encephalitis cases (4.3.2) should also shed light on the incidence of other arboviral diseases of humans in Nepal.

4.3.9 Personnel Needs for Field Studies

To carry out the proposed longitudinal field studies the following personnel will be required.

a. Director of Field Operations,
   Entomologist/Vector Ecologist
   Masters or Doctoral Level

b. Field Supervisor
Bachelors or Masters Level or appropriate experience

c. Mosquito Taxonomist
Appropriate Experience or training

d. Mosquito Scouts to collect and process specimens
Six individuals with appropriate background or training

e. Vertebrate Ecologist or Veterinarian to take major responsibility for specimen collection from vertebrates.

f. 2 Assistants for (e)

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ANNEX - I

PRINCIPAL PERSONS CONTACTED:

Nepalese Ministry of Health:

1. Mrs. Sushila Thapa - Minister of Health
2. Mr. Basudev Pradhan, Acting Secretary of Health
3. Mr. Bihari Krishna Shrestha, Additional Secretary of Health
4. Mr. M. B. Parajuli, Chief, Epidemiology Division
5. Dr. V. L. Gurubacharya, Superintendent, Central Health Laboratory (CHL)
6. Mr. Jitendra P.B. Shrestha, Acting Chief, Nepal Malaria Eradication Organization (NMEO)
7. Mr. Shreebatsha P. Shrestha, Acting Chief, Planning Division
8. Dr. Chandrika Shrestha, Virologist, CHL
9. Dr. M. K. Banerjee, Epidemiologist, NMEO

USAID:

1. Mr. William Stacy Rhodes, Deputy Director
2. Dr. David H. Calder, Chief, HFP
3. Mr. J.L. Anderson, Project Officer, HFP
4. Ms. Ursula Nadolny, Project Officer, HFP
5. Mr. Shreedhar P. Pradhan, Malaria Advisor, HFP

OTHERS:

1. Dr. P.N. Sehgal, World Health Organization, Short-Term Consultant to Nepal MOH on Meningitis and Japanese Encephalitis

ANNEX II

Consolidated Data of Japanese Encephalitis Case Incidence Recorded in the Year 1988 in Nepal

REGION	DISTRICT	TOTAL CASES	TOTAL DEATHS	CASE FATALITY RATE.
East	Jhapa	57	9	15.78%
	Morang	100	11	11.00%
	Sunsari	68	15	22.05%
	Udayapur	17	3	17.64%
	Saptari	41	21	51.21%
	Siraha	13	4	30.76%
		296	63	21.28%
Central	Dhanusa	36	11	30.55%
	Mahottari	19	8	42.10%
	Sarlahi	10	0	0.0%
	Bara	51	19	37.25%
	Parsa	44	13	29.95%
	Rautahat	5	1	20.00%
	Makawanpur	6	1	16.66%
	Chitwan	10	5	50.00%
		181	58	32.04%
West	Nawalparasi	80	37	46.25%
	Rupendahi	173	73	42.19%
	Kapilbastu	113	24	21.23%
	Palpa	11	3	27.27%
		377	137	36.33%
Mid-West	Dangdeokhuri	67	22	32.83%
	Bankey	170	31	18.23%
	Bardia	188	53	28.19%
		425	106	24.94%
Far-West	Kailali	74	21	28.37%
	Kanchanpur	25	12	48.00%
		99	33	33.33%
TOTAL		1,371	397	28.95%

ANNEX III

AGE AND SEX DISTRIBUTION OF VIRAL ENCEPHALITIS (JE) ADMITTED AND TREATED IN HOSPITAL OF PLAIN TERAI AND INNER TERAI BELT OF NEPAL YEAR - 1988

Age Group	Female					Male							
	Total Cases	Total Deaths	Morta-lity	Un-known	Lama	Total Cases	Total Deaths	Morta-lity	Un-known	Lama	Total Cases	Total Deaths	Morta lity
0-12 Months	5	0	0.0%	0	2	5	2	40.0%	0	1	9	2	22.22
1-4 Years	69	24	34.78%	9	5	109	32	29.35%	15	15	180	56	31.11
5-9 Years	144	45	31.25%	20	19	224	56	25.0%	27	41	368	101	27.44
10-14 Years	96	29	30.20%	7	14	152	41	26.97%	21	22	248	70	28.22
15 + Above	236	52	22.03%	47	30	313	107	34.18%	34	39	549	159	28.96
Total Unknown age	11	3	27.27%	8	0	7	6	85.71%	0	1	18	9	50.0%
Total	561	153	27.27%	91	70	810	244	30.12%	96	119	1371	397	28.95

ANNEX - IV

Equipment Needed for Arbovirus Laboratory in Nepal:

Revco Freezer

Voltage Regulator for Revco
Model ULT 1285B

Extra compressor for Revco

- 20°C Freezer

Laminar Flow Cabinet
Biohazard BBL Class II

Base for Cabinet

Autoclave--electric not computerized
chamber at least 36 x 20 x 20

Extra heating elements for autoclave

Water purification system

Incubator---double door

Incubator CO_2

Dynatech Elisa reader

PH meter

Water bath

Vibrator--Vertical

4 Liquid Nitrogen Tanks
Model LD31

2 Vortex mixers, Genie

Magnetic Stirrer

Stirring Bars

Refrigerated Incubator

Florescent Microscope---Olympus with epi-illumination

Inverted Microscope Olympus

4 air conditioners, 220V 18,000 BTU

PR 6000 refrigerated centrifuge with accessories

IBM---AT computer with printer
word processor
software:
database III+
lotus (latest version)
wordperfect

Microtiter, Micropipette, reusable
0.050 ml
0.025 ml

Microtiter, microdiluter
0.025 ml
0.05 ml

Microtiter plates, polystryene
rigid V plates--disposable

Microtiter mirror reader

Microtiter go-no-go testers
for .05 and 0.025 ml

Glassware

Chemicals

Consumable supplies

ANNEX V

Terai Trip Report of Dr. Mitchell: January 31 - February 4, 1989

Itinerary:		
	January 31	Kathmandu to Bhairahawa
	February 1	Bhairahawa to Butwal to Hetauda
	2	Hetauda to Biratnagar
	3	Biratnagar to Hetauda
	4	Hetauda to Kathmandu

The trip was made in a U.S. Agency for International Development (USAID) vehicle. I was accompanied by Mr. Shreedhar P. Pradhan, USAID Malaria Advisor, and Dr. M. K. Banerjee, Epidemiologist, Nepal Malaria Eradication Organization.

Principal Persons Met:

In Butwal:

Dr. S. Pahari, Medical Superintendent, Lumbini Zonal Hospital
Dr. T. Rajbhaudari, Pathologist, Lumbini Zonal Hospital

In Biratnagar:

Mr. Basudev Pradhan, Acting Secretary of Health, Kathmandu (visiting Biratnagar)
Dr. K.R. Panday, Regional Director, Eastern Region, NMEO
Dr. R. Sinha, Medical Superintendent, Koshi Zonal Hospital
Mr. R.P. Ghimire, Chairman, Koshi Zonal Hospital Board
Dr. G.M. Bajracharya, Pathologist; Dr. B. Bataya, Pediatrician; and
Dr. K. Malla, Gynecologist; Koshi Zonal Hospital
Mr. J.D. Shrestha, Chief Public Health Officer, NMEO, Eastern Region

On Wednesday morning we visited the Zonal Hospital in Butwal and met the hospital staff. I gave a brief presentation about the ecology and epidemiology of Japanese encephalitis (JE) and reiterated our short-term recommendations and long-term plans as outlined in the main body of our report. We visited the pathology laboratory, which does routine biochemical tests, but has no facilities for laboratory diagnosis of JE.

We then visited the village of Shantinagar from which clinical cases of viral encephalitis have been reported during the past several years. The area is semi-rural with paddy fields bordering the village. Large piles of rice straw are common and would make excellent sites for collecting engorged, resting mosquitoes for host preference studies. Swine are commonly found

throughout the village, both free roaming and restrained in crude enclosures. In the immediate vicinity, cattle egrets, crows, ducks, sparrows, water buffalo, dogs and horses were commonly sighted.

We visited the Police Station in Shantinagar. This station is used for training recruits and also rears a small number of pigs for food. Therefore, the possibility exists for conducting sero-surveys among men and swine before and after the peak JE transmission season. The officer-in-charge was very cooperative and reported that one of his staff had viral encephalitis during 1988.

The trip to Biratnagar from Bhairahawa illustrated the difficulty in attempting to control JE in the terai through vector control. There are some discrete hamlets, but human habitations are more or less continuous along the fringe of the highway and are interspersed throughout the paddy field areas. This make area control of vector mosquitoes virtually impossible using current technologies.

There are extensive fresh water swamps bordering the Saptakosi River above the dam near Bahamjia in Saptari and Sunsari Districts. Since the Sunsari District borders the Morang District on the west, and in view of the JE problem in the Morang District, these swamps appear to be excellent habitats for enzootic transmission of JE virus between vector mosquitoes and water fowl.

In Biratnagar, I repeated the presentation about JE to health officers and officials listed above. Mr. Pradhan, Acting Secretary of Health, was visiting from Kathmandu and also sat in on the discussions. He was very positive about our proposed program and indicated that appropriate slots for training would be made available according to our earlier recommendations. We visited the Zonal Hospital and saw the facilities there. Their 150-bed hospital receives a great strain on resources during epidemics of viral encephalitis. Frequently, additional beds and cots must be placed in corridors to accommodate patients.

On the return trip to Kathmandu we stopped briefly at the Ministry of Agriculture facility north of Biratnagar. They have pig-rearing facilities and their swine could be used as sentinels for JE surveillance in that area. Cases of JE in humans reportedly have occurred in the adjacent village.

Recommendations

1. Future investigations should include study sites in Shantinager, Lumbini Zone, and at an additional site in the vicinity of Biratnagar, Koshi Zone.

2. The swamps adjacent to the Saptakosi River should be considered as prime candidates as foci for enzootic maintenance of JE virus in Nepal, or as sites for reintroduction of the virus by migratory birds.

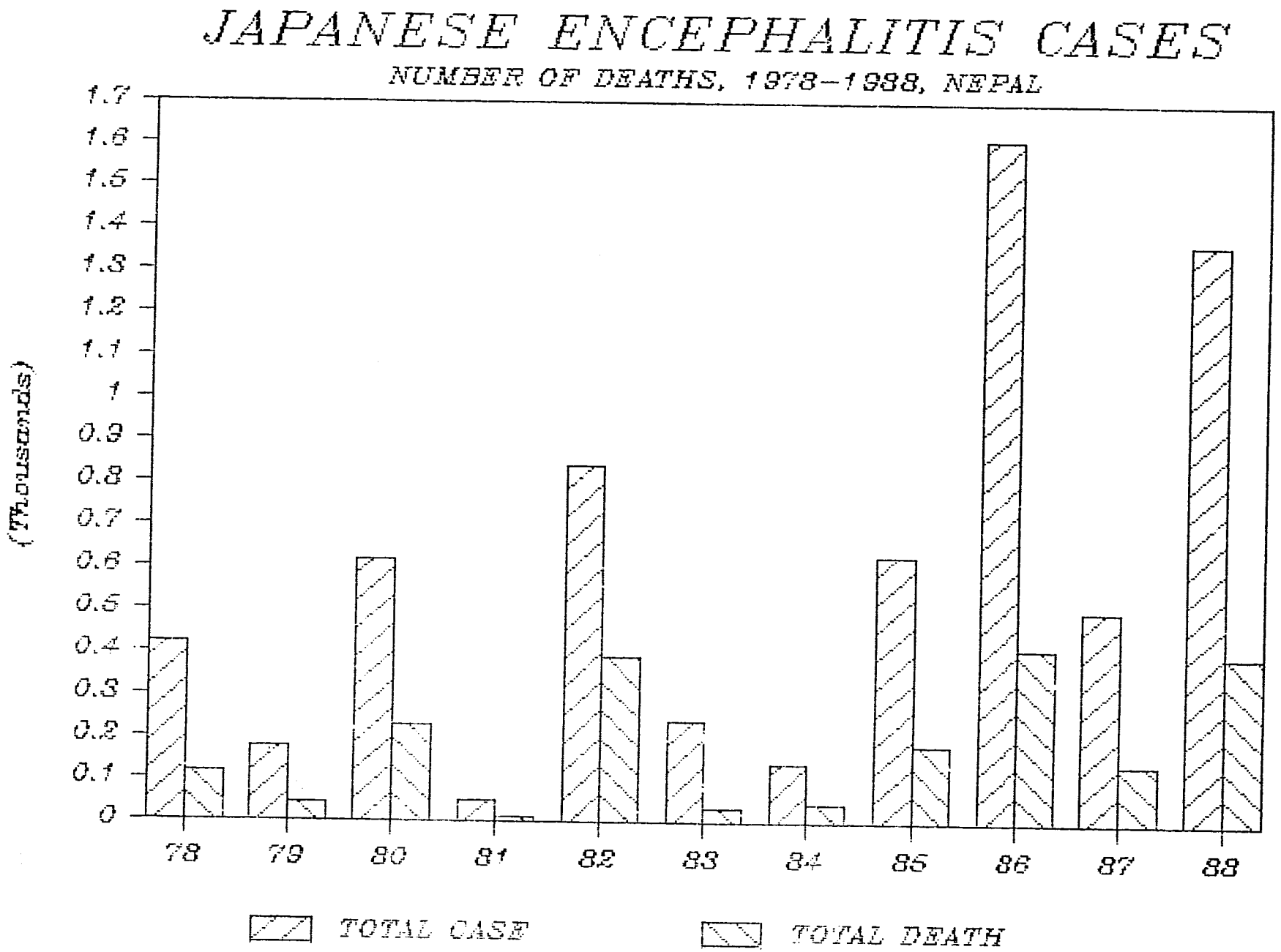

JAPANESE ENCEPHALITIS CASES
NUMBER OF DEATHS, 1978-1988, NEPAL
(Thousands)
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
78
79
80
81
82
83
84
85
86
87
88
TOTAL CASE
TOTAL DEATH

Figure - 1

NEPAL

SHOWING ENCEPHALITIS ENDEMIC DISTRICTS

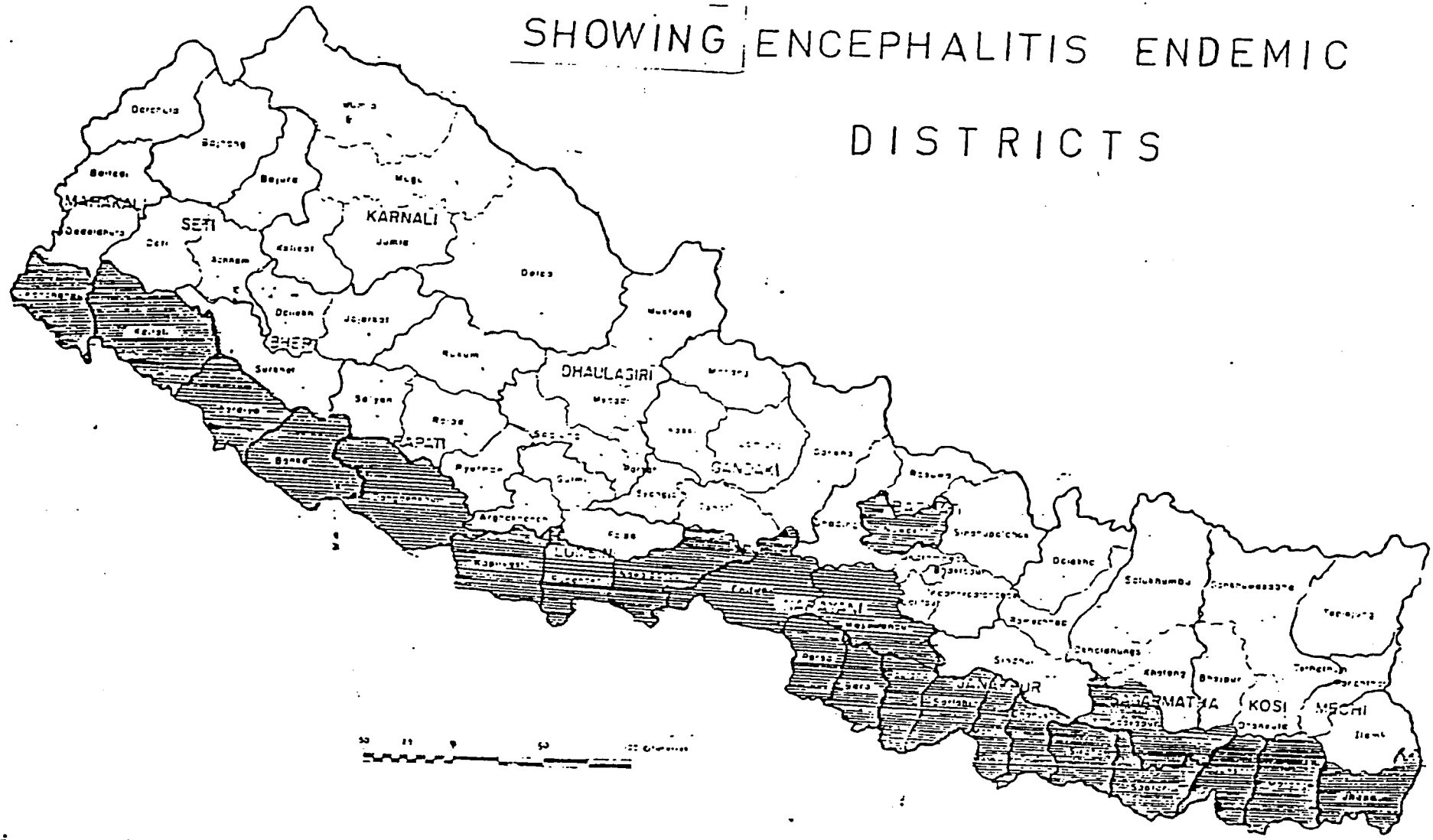


Figure - 2

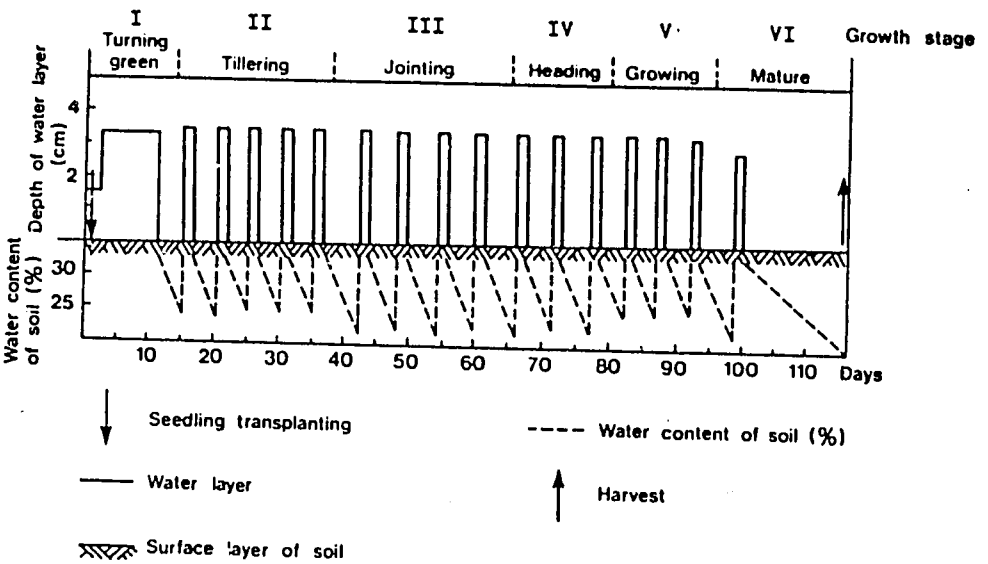
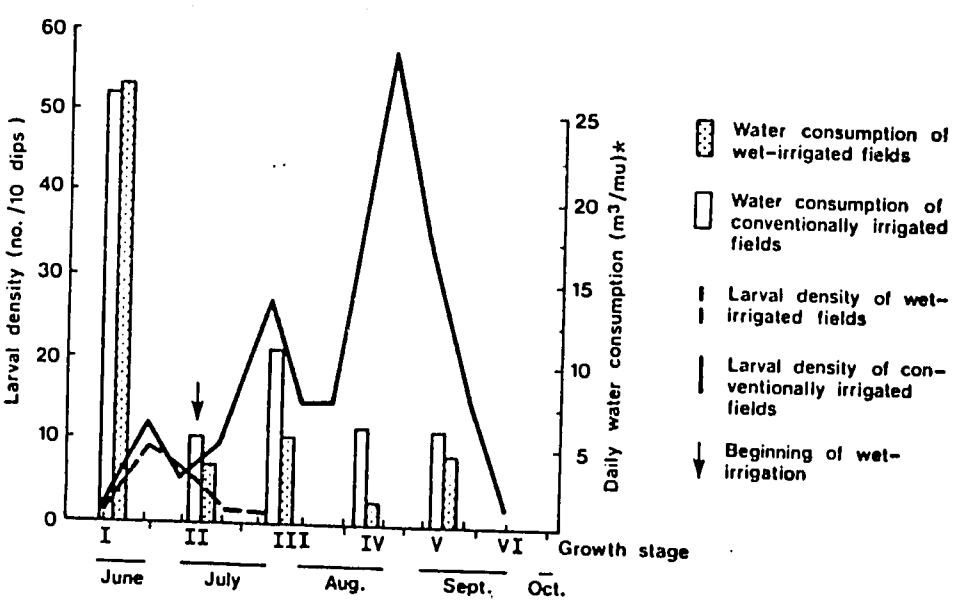


Fig. 3 Diagram of wet irrigation method in rice cultivation in Henan Province (after Ge Fengxiang *et al.* 1981)



* 15 mu = 1 ha

Fig. 4 Comparison of the larval densities of *An. sinensis* and *Cx. tritaeniorhynchus* between fields of wet and conventional irrigation (after Ge Fengxiang *et al.* 1981)