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SCIENTIFIC NOTE

STUDIES ON DENGUE IN RURAL AREAS OF KURNOOL DISTRICT, ANDHRA PRADESH, INDIA

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ABSTRACT. A dengue case was reported for the 1st time in a rural area of Kurnool District, Andhra Pradesh, India. Entomological and serological investigations were carried out to determine the prevalence of dengue vectors and dengue virus. *Aedes aegypti* was recorded for the 1st time in rural areas of Andhra Pradesh. Breeding of *Ae. aegypti* was observed only in containers with nonpotable water. Cement cisterns and tanks, stone tubs, and clay pots were the major breeding habitats of *Ae. aegypti.* Larval indices for *Ae. aegypti* ranged as follows: house index 28–40%, container index 13–37%, and Breteau index 32–60. A serological survey indicated that humans in Kurnool District have been exposed to dengue virus infections. The potential threat of an outbreak of dengue fever in rural areas because of the prevalence of the vector (*Ae. aegypti*) and dengue virus is discussed.

KEY WORDS Dengue, *Aedes aegypti,* house index, container index, Breteau index, Kurnool

Dengue fever (DF) and its severe forms, dengue hemorrhagic fever and dengue shock syndrome, are mosquito-transmitted arboviral diseases belonging to genus *Flavivirus,* family *Flaviviridae.* Dengue fever is the most important arboviral disease of humans and its control is dependent on managing populations of *Aedes aegypti* (L.) (Gubler 1989). Dengue fever outbreaks have mainly been reported from urban areas of India. However, a few outbreaks in the recent past also have been reported from rural areas of Maharashtra (Ilkal et al. 1991, Mahendale et al. 1991), Gujarat (Mahadev et al. 1993), Haryana (Katyal et al. 1997, Avdesh Kumar et al. 2001), and Tamil Nadu (Abdul Kader et al. 1997). In Andhra Pradesh, only a few DF cases were reported in urban areas until 2001. However, in September 2002, DF cases were reported from Hyderabad, Warangal, and Kurnool districts. In Kurnool District, 1 DF case (a girl, aged 9 years, had hemagglutination inhibiting antibodies against dengue-2 antigen with a 1:160 titer) was reported from a rural area for the 1st time. Vector control is the only option available for dengue prevention and control. Thus, assessment of breeding sources of *Aedes* is important for devising suitable control programs. Hence, preliminary entomological and serological surveys were carried out in rural areas of Kurnool District to determine the prevalence of dengue vectors and their breeding habitats as well as the presence of dengue virus.

Three villages (Halakarvi, Kamnehal, and Nandanapalli) in Kurnool District (Fig. 1) were selected for the entomological survey. Halakarvi was selected because of a confirmed case of dengue from this village. Kamnehal was selected because more fever cases were reported from this village than from others during the study period (September 2002). Nandanapalli was selected based on the results of a serological survey conducted in June 2002 (14% of school children in this village were mono-specific positive against dengue virus antigen).

In the study villages, domestic animals such as cattle, pigs, and poultry are common and are housed near human habitation. People in these villages belonged to an economically poorer group and generally lived in thatched huts. Tube wells are the principal water source for the study villages. In almost all houses, water for washing and bathing is stored in cemented containers (cement cisterns, cement tanks, and clay pots). The villagers use plastic and metal containers for storing potable water.

Houses were surveyed for breeding of *Aedes* in water-storage containers by using a single larva survey method (Sheppard 1969) and the larval density was expressed as house index (HI), container index (CI), and Breteau index (BI, number of positive containers per 100 houses). Adult female *Ae. aegypti* resting indoors were collected by using a flashlight and suction tube. Eight houses were selected randomly in each study area and 2 insect collectors covered the houses in an hour. The resting density was expressed as number of female *Ae. aegypti* collected per man-hour. Landing collections also were conducted indoors during the daytime. The mosquitoes landing on a human volunteer (1 of the members of our team or a member of the household in which mosquitoes were collected) were collected and a total of 8 randomly selected houses were covered in 2 h by 2 insect collectors in each study area. The

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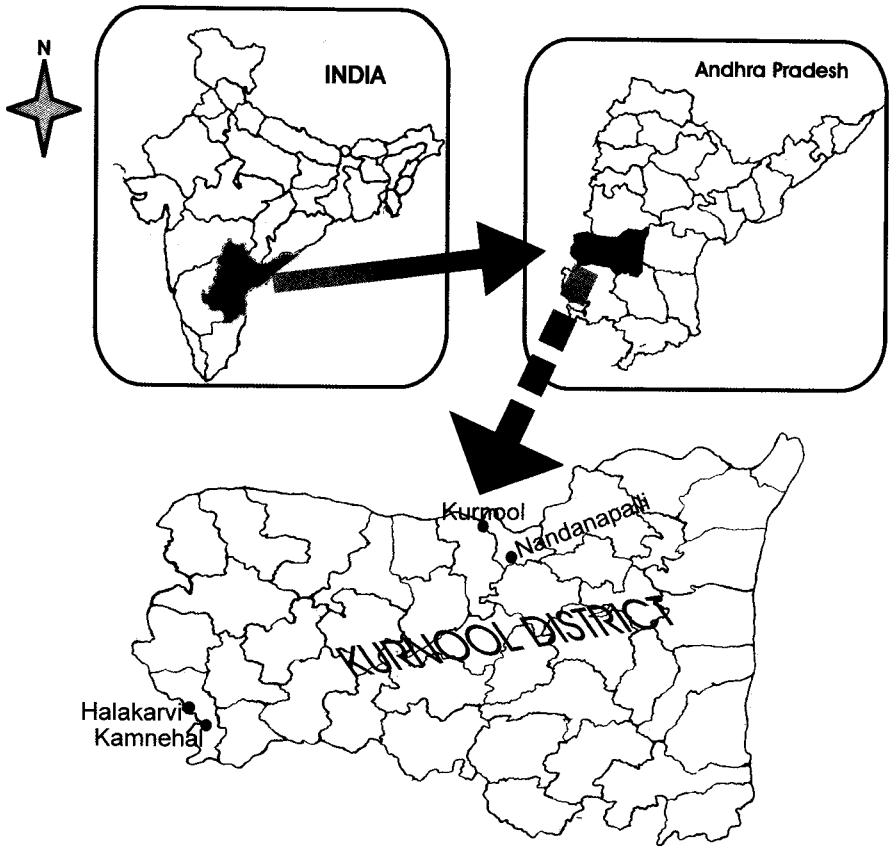

Fig. 1. Map of Kurnool District, Andhra Pradesh, India, with study sites indicated.

landing density is expressed as number of female mosquitoes collected at bait per hour.

Serum samples were collected from primary school children after obtaining informed consent from the parent or guardian. The student population was selected for the serological survey to represent the entire village. The Institutional Ethical Committee of Centre for Research in Medical Entomology approved the study protocol. Serum samples also were collected from persons suspected of having dengue. Samples were stored in a refrigerator until they were transported to the laboratory on wet ice. These sera were subjected to a hemagglutination inhibition test to ascertain the extent of the flavivirus infections in the villages (Clarke and Casals 1958). Adult *Ae. aegypti* were pooled by village and were transported in liquid nitrogen to the laboratory for detection of dengue antigen by immunofluorescence assay (Gajanana et al. 1995).

Domestic water storage containers contribute to extensive breeding of *Ae. aegypti* in rural and urban areas. Cement cisterns and tanks, stone tubs, and clay pots were the major breeding habitats of *Ae. aegypti* (Table 1). Stone tubs are typical domestic water-storage containers in rural areas. Although

Table 1. Breeding of *Aedes aegypti* in domestic containers in Kurnool District.

	Rural			Urban		
Type of container	Number surveyed	Number positive	% positive	Number surveyed	Number positive	% positive
Cement cistern	31	10	32.3	34	13	38.2
Cement tank	21	5	23.8	26	13	50.0
Metal container	23	0	0.0	8	0	0.0
Clay pot	33	3	9.1	52	17	32.7
Plastic container	35	0	0.0	40	0	0.0
Stone tub	16	11	68.8	—	—	—

plastic and metal containers, which are used mainly for storing potable water, were found in large numbers, no breeding of *Aedes* was observed in these containers.

Adult mosquito (landing and resting) collection was restricted to only 8 houses selected randomly for the preliminary study because collecting adult *Aedes* was a cumbersome exercise. Keeping this view, limited effort was made in adult mosquito collection, whereas more emphasis was given for the larval survey and a total of 113 houses were surveyed from the study areas for the infestation with larval *Aedes*. The HI in the villages ranged from 28% to 40%. The highest HI (40%) was recorded in Halakarvi. The CI index ranged from 13% to 37%. The highest BI (60) was recorded in Kamnehal, whereas in Halakarvi and Nandanapalli, BIs were 48 and 32, respectively. Surveys for *Aedes* also were conducted in Kurnool town (urban) for comparison with rural areas. Larval indices for *Ae. aegypti* were higher in urban areas compared to villages. In Kurnool urban area, the HI was 58% and the BI was 90.

Landing collection was conducted in Halakarvi and the landing density of *Ae. aegypti* was 2.5 females/man-hour. Resting collection in the same village yielded 5 female *Ae. aegypti*/man-hour. Sixty adult *Ae. aegypti* in 10 pools were tested for dengue virus infection and all pools were found to be negative.

A cross-sectional sera survey of school children was carried out to determine the prevalence of dengue virus infection and 49 finger-prick blood samples were collected from school children aged 6 to 10 years from Halakarvi, where a confirmed dengue case was reported. The samples were tested by a hemagglutination inhibition test and flavivirus antibody reactivity was seen in 14 samples (28.6%) of the sera. Five samples (10.2%) were mono-specific positive for Japanese encephalitis virus (JE) and 1 sample (2.0%) was mono-specific positive for West Nile virus (WN), but 8 samples (16.3%) from children aged 7–10 years were positive for more than 1 flavivirus, including dengue virus. In Kamnehal, 23 sera were collected from people with fever from various age groups (aged 5–58 years). Eight of 9 positive samples from people aged 8–56 years were positive for JE, WN, and dengue virus. One sample was mono-specific positive for JE.

Clay pots, cement cisterns and tanks, and stone tubs were found as the major breeding habitats of *Ae. aegypti*. Thoroughly cleaning and replenishing cement containers and stone tubs is very inconvenient, and these habitats form ideal breeding sites for *Ae. aegypti*. Breeding of *Ae. aegypti* was predominantly observed in containers with nonpotable water, which agrees with earlier findings that potential breeding by *Aedes* was lower in potable water storage containers than in nonpotable water storage containers (Mahadev et al. 1993). The results of the study are of practical significance to a DF control program in Kurnool District. Containers such as clay pots, cement cisterns, and tanks stone tubs were infested with *Ae. aegypti* and they should be given high priority in DF control (source-reduction) programs.

A sera survey of children was carried out in rural areas of Kurnool District to determine the prevalence of flavivirus. The serological (hemagglutination inhibition) test used in this study is a useful diagnostic tool but is time consuming. Gajanana et al. (1996) compared the hemagglutination inhibition test with a virus isolation technique (*Toxorhynchites splendens* inoculation) and equal sensitivity and negative predictive values were found in both tests. In 1 of the study villages (Nandanapalli), 7 (14%) of 50 samples were unambiguous against dengue virus antigen. However, no mono-specific dengue positive was observed in the other 2 study villages. However, a mixed reaction (including dengue virus) was observed. The results suggest that the human population in rural areas of Kurnool District was exposed to dengue virus infections.

Outbreaks of DF have been reported from the rural areas of various states of India because of the invasion of *Ae. aegypti* in rural areas. This is the 1st report of the prevalence of *Ae. aegypti* and dengue virus in rural areas of Andhra Pradesh. Ilkal et al. (1991) postulated that the spread of *Ae. aegypti* to rural areas was contributed to by better transportation facilities and supply of drinking water through taps, which compels villagers to store water because the supply either was irregular or was available for a short duration. Breeding of *Ae. aegypti* was observed in tire-vulcanizing shops in rural areas of Gujarat (Mahadev et al. 1993). The transportation of used tires was cited as the principal cause for the widespread distribution of this species (Reiter and Sprenger 1987). The extensive movement of people could be the reason for the spread of dengue virus. However, unless such spread is accompanied by prevalence of the vector, the disease would become self-limiting, and an epidemic cannot occur (Mahadev et al. 1993). Indices for *Ae. aegypti* in the study villages are comparable with those in the Kurnool urban area, which shows the magnitude of the invasion of *Ae. aegypti* in the rural areas. Invasion of *Ae. aegypti* in rural areas of Kurnool District and the prevalence of dengue virus in the rural biotope indicate the possibility of a potential dengue outbreak.

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