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Short Research Communications

Establishment of a focus on *Anopheles fluviatilis,* an important malaria vector near the National Thermal Power Corporation Project in Dadri CHC area in District Gautam Budh Nagar, Uttar Pradesh, India: A case study

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Malaria is one of the major public health problems in rural plain areas of India, where *Anopheles culicifacies* Giles (Diptera: Culicidae) is the primary malaria vector species. This species is responsible for about 60–65% of all reported malaria cases in India[1] that breeds in irrigation channels, ponds, pools and rice fields. *Anopheles fluviatilis* James, is another important vector of malaria in India, which is found specifically in foothills and breeds in slow moving water in streams and channels. Various studies carried out in past in western Uttar Pradesh, India have reported *An. culicifacies* s.l. as the only established vector of malaria in this area[2–3], whereas *An. fluviatilis*, has never been reported from this area. *An. culicifacies* s.l. is a complex of five sibling species[4] out of which only two sibling species, viz. species A and species B are reported to be prevalent in this area. The area under Dadri Community Health Centre (CHC) in western Uttar Pradesh has remained highly endemic due to perennial breeding of *An. culicifacies* in the seepage water from irrigation channels of the Upper Ganga Canal. During 1980's a new project of the National Thermal Power Corporation (NTPC) was setup in this area to meet the demand for power supply in the National Capital Region without the health impact assessment. While carrying out a field survey in Dadri CHC area, we noticed the presence of *An. fluviatilis* in high densities for the first time during November 2009. In the present study, we report the establishment of a new focus of *An. fluviatilis* in the area and possible repercussion in connection with malaria transmission.

Dadri CHC area of District Gautam Budh Nagar in Uttar Pradesh (9° 72′N and 80° 28′E) is located at a distance of about 40–45 km from Delhi. The weather conditions can be divided into four seasons, summer (March–June), monsoon (July–August), autumn (September–October) and winter (November–February). The maximum temperature during summer months goes up to >46°C and the minimum temperature during winter in the month of January falls to <4°C.

An entomological survey was carried out in different villages of Dadri CHC during the months of October–November 2009, which revealed the prevalence of *An. fluviatilis* s.l. in some villages of Dadri CHC. All the villages (Fig. 1), from where *An. fluviatilis* were collected are located adjacent to a canal, which carries the discharged water from NTPC plant along with surplus water from the tributary of the Upper Ganga Canal. The density of *An. fluviatilis* in this area was regularly monitored in six representative villages located adjacent to NTPC canal, where *An. fluviatilis* was detected for the first time in the month of November 2009. Indoor resting adult mosquitoes were collected at monthly intervals up to September 2012. Adult anopheline mosquitoes were collected from four fixed and four randomly selected human dwellings and cattlesheds by hand catch method using a flashlight and an aspirator during the morning hours and transported in thermocol box to our laboratory for further analysis. In addition to the indoor resting adult mosquito collection, anopheline larvae were also collected periodically from NTPC canal and from other larval habitats in the area and brought to the laboratory and reared up to adult stage in the insectary. The emerged adults were identified both morphologically and by molecular method for reconfirmation of species. All the adult mosquitoes were identified morphologically using standard identification key[5]. Morphologically identified *An. culicifacies* and *An. fluviatilis* were further processed for the sibling species composition by cytotaxonomy and DNA sequence[6–8].

Cytolological identification of *An. culicifacies* and *An. fluviatilis* sibling species was carried out by examining the ovarian polytene chromosomes for species-specific diagnostic inversions for the identification of the

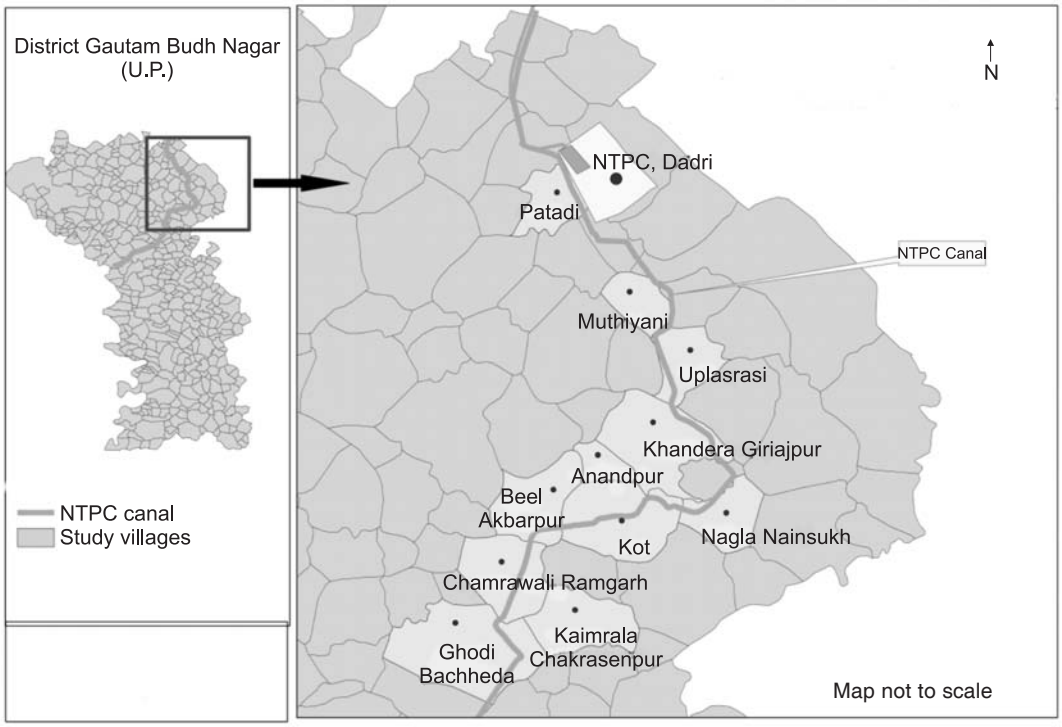

Fig. 1: Map of District Gautam Budh Nagar (U.P.) and the locations of villages from where *An. fluviatilis* was recorded.

members of *An. culicifacies*[6] and *An. fluviatilis*[7] complexes. Identification of *An. fluviatilis* sibling species was reconfirmed by sequencing D3 domain of 28S rDNA following the method described by Singh *et al*[8]. The blood meal source identification was carried out using fed mosquitoes by counter current immunoelectrophoresis[9].

Collection of mosquitoes in the six study villages revealed the prevalence of *An. culicifacies* throughout the study period, except during the winter months of January and February, when the density was almost negligible. The peak mosquito density of *An. culicifacies* was recorded from July to September in each year (Fig. 2). These

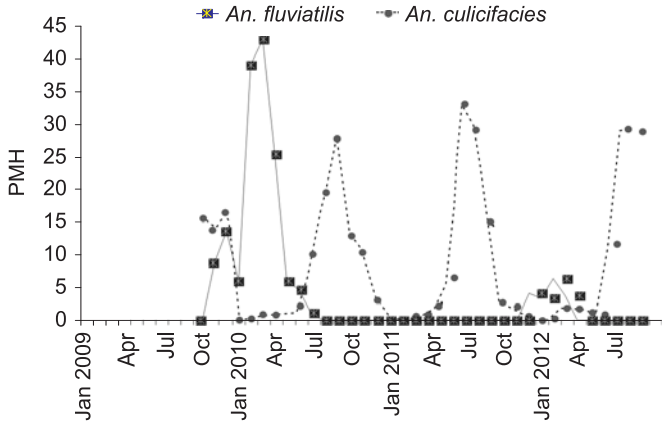

Fig. 2: Monthly indoor resting density of *Anopheles culicifacies* and *An. fluviatilis* in Dadri CHC area during October 2009 to September 2012.

observations indicate a seasonal change in the density of *An. culicifacies* population during each year. On the other hand *An. fluviatilis* were collected only during November 2009 to July 2010 and again during January to April 2012 (Fig. 2). No specimen of *An. fluviatilis* could be collected from any of these villages during the months of August 2010 to December 2011 and further during the month of May to September 2012. It may be mentioned that unlike *An. culicifacies* there was no seasonal changes in the density of *An. fluviatilis* population.

Most important observation made from the study was that the appearance and disappearance of *An. fluviatilis* which was correlated with the presence and absence of thick water hyacinth vegetation cover in the NTPC canal (Fig. 3a & b). The water hyacinth was later removed manually throughout the length of NTPC canal during June–July 2010. The vegetation cover again reappeared during December 2011, which was again removed during March 2012. It may be mentioned that NTPC canal carries discharged water from Thermal Power Plant after cooling of towers and ash effluents. This water is taken from irrigation channel, a tributary of Upper Ganga canal, which receives water from the River Ganga. It may be mentioned that this river passes through the Himalayan foothills and *Terai* region, where *An. fluviatilis* is found in abundance[10–11]. Earlier studies have incriminated *An. fluviatilis* as one of the malaria vectors in *Terai* area[12].

NTPC is located in the command area of Upper Ganga

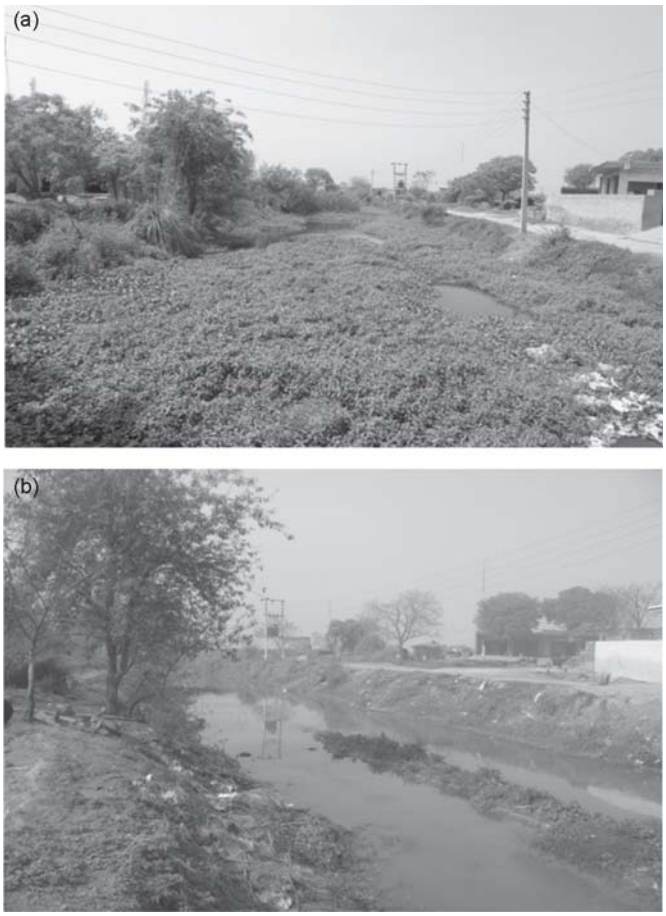

Fig. 3(a & b): NTPC canal showing: (a) vegetation cover; and (b) after removal of vegetation cover.

Canal, a water logged area due to persistent seepages from adjoining irrigation channels and responsible for proliferation of mosquito breeding, particularly of *An. culicifacies*. Outbreaks of malaria have been reported during the initial phase of construction of thermal power plant in 1986 in this area, but *An. fluviatilis* was never reported from this area earlier[3].

In the present study, a total of 503 *An. culicifacies* s.l. and 747 *An. fluviatilis* were collected during the year 2010. All the *An. fluviatilis* specimens (n = 66) examined were identified as species T of the *An. fluviatilis* complex and were monomorphic for q^1 inversion. Representative samples (n = 10) that were sequenced for D3 domain of 28S rDNA were found to be 100% homologous to *An. fluviatilis* species T. Blood meal source analyses revealed that all *An. fluviatilis* (n = 66) were zoophagic. *Anopheles fluviatilis* in India is a complex of four sibling species, viz. S, T, U and V[13]. Cytological and molecular diagnostic techniques have confirmed that the *An. fluviatilis* found in Dadri area is sibling species T and is primarily zoophagic.

The appearance of *An. fluviatilis* species T in Dadri CHC area might have occurred by transportation of larval population through the tributary of Upper Ganga Canal, which carry water from foothills and *Terai* areas of Uttar Pradesh (now in Uttrakhand state), where *An. fluviatilis* species T and U have been reported[10–11]. This had facilitated the establishment of *An. fluviatilis* population in NTPC canal, which was under the cover of thick vegetation. The appearance and subsequent disappearance of *An. fluviatilis* has a likely association with the presence and absence of thick vegetation on the surface of slow moving water in the NTPC canal. It may also be pointed out that although larval collections were made periodically from NTPC canal and from other breeding habitats present in the study area, *An. fluviatilis* was found to be breeding in the NTPC canal only and not in any other larval habitats in the nearby areas, which could probably be due to slow moving water in the NTPC canal. The establishment of *An. fluviatilis* in the NTPC command area suggests that health impact assessment of development projects should be undertaken to prevent creation of mosquitogenic conditions in malaria endemic areas.

Cytological examination of *An. culicifacies* populations from the same area revealed that species A and B were prevalent in the study villages with predominance of species A (73%), and were found to be primarily zoophagic. The sibling species of *An. culicifacies* are reported to show different ecological and biological characteristics which influence susceptibility to parasites and insecticides in India[6]. Dadri CHC area in Gautam Budh Nagar, U.P. has been reported to be endemic area for malaria. *Plasmodium vivax* is the predominant malarial parasite followed by *P. falciparum.* Two peaks of malaria incidences are observed in this area[3]. However, there has been a reduction in malaria incidence in this area during last few years which might be due to urbanization in the area and related ecological changes.

Regarding the role of *An. fluviatilis* in malaria transmission it may be pointed out that out of four sibling species of Fluviatilis Complex (species S, T, U and V), only species S has been implicated as a major malaria vector in India[13–15] and species T and U are considered as poor vectors. However, recent field studies have revealed that the species T is also playing a role in the transmission of malaria in Jharkhand and Madhya Pradesh states in the country[16–17].

Absence of accurate knowledge concerning the ecology of vector species has been responsible for our inadequate understanding of epidemiology of malaria. The longitudinal study will open avenues to study mosquito ecology, particularly dynamics of ecological changes and

their impact on malaria transmission. In depth study will provide new insight in understanding the ecological succession of new mosquito vector species in this area.

The present study shows establishment of *An. fluviatilis* population in a completely new area from where this species has never been reported earlier, due to the presence of a suitable breeding habitat. The most interesting observation was the linked association of appearance of new mosquito species, i.e. *An. fluviatilis* with the presence of vegetation in slow moving water in the canal. In view of the recent incrimination of *An. fluviatilis* species T, we should keep a watchful eye on the appearance of this species and explore its probable role in malaria transmission in the area of its establishment for undertaking suitable vector control measures.

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REFERENCES

1. Sharma VP. Roll back malaria. *Curr Sci* 1998; *75*(8): 756–7.
2. Sharma VP, Chandrahas RK, Ansari MA, Srivastava PK, Razdan RK, Batra CP, *et al.* Impact of DDT and HCH spraying on malaria transmission in villages with DDT and HCH resistant *Anopheles culicifacies*. *Indian J Malariol* 1986; *23:* 27–38.
3. Ansari MA, Sharma VP, Razdan RK, Mittal PK. Field evaluation of deltamethrin against resistant *Anopheles culicifacies* in Distt. Ghaziabad (U.P.), India. *Indian J Malariol* 1990; *27:* 1–13.
4. Subbarao SK. Anopheline species complex in South-East Asia. New Delhi: WHO Regional Office for South-East Asia 1998.
5. Wattal BL, Kalra NL. Region-wise pictorial keys to the female Indian *Anopheles*. *Bull Natl Soc India Mal Mosq Dis* 1961; *9*(2): 85–135.
6. Subbarao SK. The *Anopheles culicifacies* complex and control of malaria. *Parasitol Today* 1988; *4:* 72–5.
7. Subbarao SK, Nanda N, Vasantha K, Dua VK, Malhotra MS, Yadav RS, Sharma VP. Cytogenetic evidence for three sibling species in *Anopheles fluviatilis*. *Ann Entomol Soc Am* 1994; *87:* 116–21.
8. Singh OP, Chandra D, Nanda N, Raghavendra K, Sunil S, Sharma SK, *et al*. Differentiation of members of the *Anopheles fluviatilis* species complex by an allele-specific polymerase chain reaction based on 28S ribosomal DNA sequences. *Am J Trop Med Hyg* 2004; *70:* 27–32.
9. Nanda N, Joshi H, Subbarao SK, Yadav RS, Shukla RP, Dua VK, *et al. Anopheles fluviatilis* complex: Host feeding patterns of species S, T, and U. *J Am Mosq Control Assoc* 1996; *12:* 147–9.
10. Sharma SK, Nanda N, Dua VK, Joshi H, Subbarao SK, Sharma VP. Studies on the bionomics of *Anopheles fluviatilis sensu lato* and the sibiling species composition in the foothills of Shiwalik range (Uttar Pradesh), India. *South East Asian J Trop Med Parasitol* 1995; *26:* 566–72.
11. Shukla RP, Nanda N, Pandey AC, Kohli VK, Joshi H, Subbarao SK. Studies on bionomics of *Anopheles fluviatilis* and its sibling species in Nainital district, U.P. *Indian J Malariol* 1998; *35*(2): 41–7.
12. Choudhury DS, Malhotra MS, Shukla RP, Ghosh SK, Sharma VP. Resurgence of malaria in Gadarpur PHC, District Nainital, Uttar Pradesh. *Indian J Malariol* 1983; *20:* 49–58.
13. Nanda N, Singh OP, Dua VK, Pandey AC, Nagpal BN, Adak T, *et al*. Population cytogenetic and molecular evidence for existence of a new species in *Anopheles fluviatilis* complex (Diptera: Culicidae). *Infect Genet Evol* 2013; *13:* 218–23.
14. Sharma SK, Tyagi PK, Padhan K, Upadhyay AK, Haque MA, Nanda N, *et al*. Epidemiology of malaria transmission in forest and plain ecotype villages in Sundargarh district, Orissa, India. *Trans R Soc Trop Med Hyg* 2006; *100:* 917–25.
15. Nanda N, Bhatt RM, Sharma SN, Rana PK, Kar NP, Sharma A, *et al*. Prevalence and incrimination of *Anopheles fluviatilis* species S (Diptera: Culicidae) in a malaria endemic forest area of Chhattisgarh state, central India. *Parasit Vectors* 2012; *5:* 215.
16. Annual Report 2009-10. Science and Technology Project on Integrated Disease Vector Control. Delhi, India: National Institute of Malaria Research 2010; p. 86.
17. Annual Report 2010-11. Science and Technology Project on Integrated Disease Vector Control. Delhi, India: National Institute of Malaria Research 2011; p. 89.

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