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Density, distribution, and habitat association of *Culex tritaeniorhynchus* in rural regions of Rajkot District, Gujarat, India

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Abstract
An extensive study was conducted to relate seasons, for field survey and sample collection were attempted for the first time in the rural villages area surrounding Rajkot City to explore and investigate the breeding and resting habitat preferences and distribution pattern of *Culex tritaeniorhynchus* species. Seven villages were identified for the Species identification, larval as well as adult density, and distribution of breeding sites of the species were studied in the rural villages. Results show that the highest density of adult *Culex tritaeniorhynchus* was recorded in the monsoon, while post-monsoon was most favourable to breeding in this region, and starts decreasing density during winter, causing the temporary breeding site to dry out because no precipitation then reaches the lowest density during hot post-summer seasons. Minimum density was recorded in April-May because of the temperature increase and temporary breeding sites drying out. It was clearly indicated that climatic conditions (temperature, rainfall, humidity, etc.), natural and manmade breeding sites play a vital role in the density and distribution.

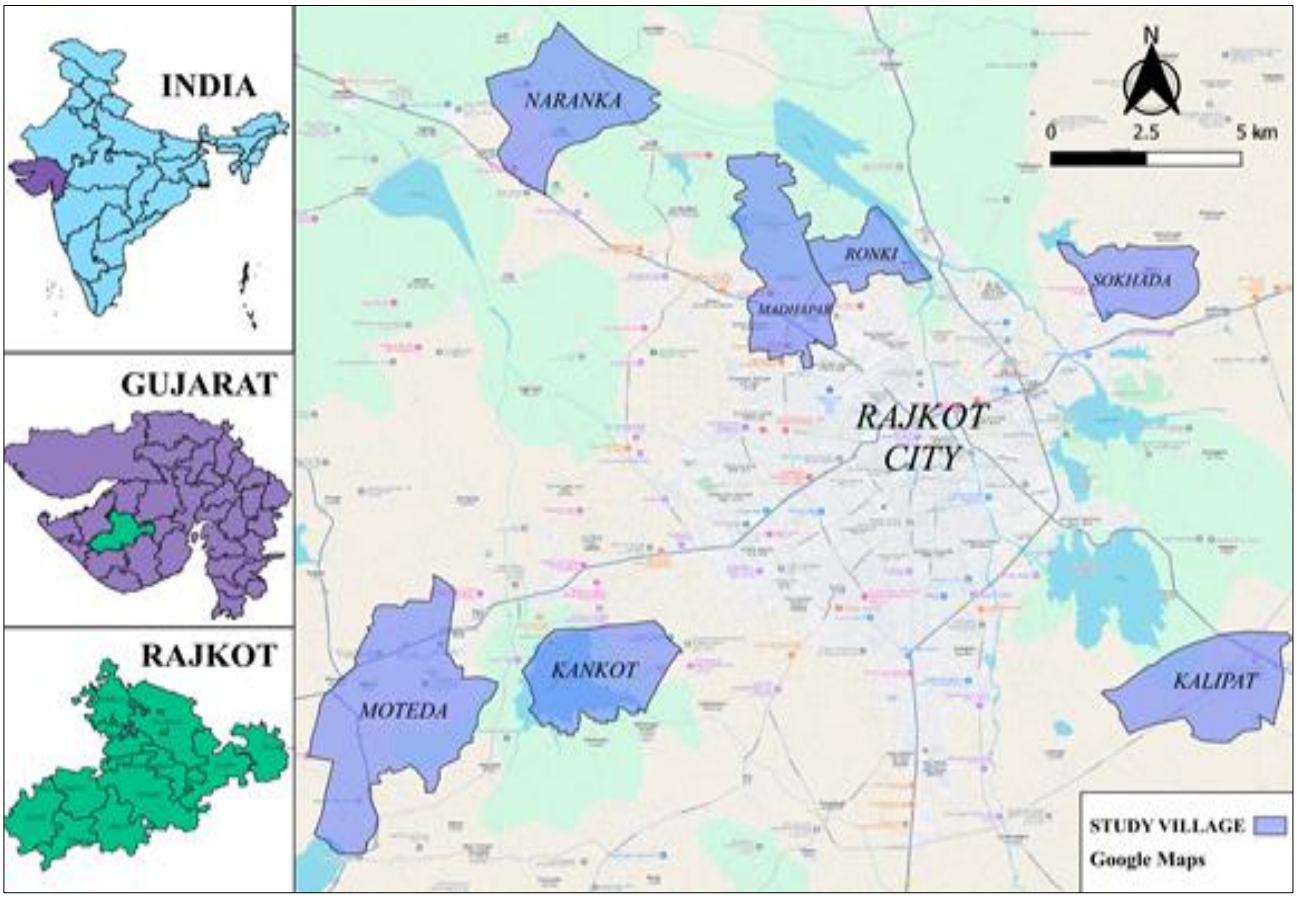
Keywords: *Culex tritaeniorhynchus* monsoon seasons, Rajkot, breeding, rural

1. Introduction
The mosquito species *Culex* (*Culex) tritaeniorhynchus* is the primary carrier of Japanese encephalitis. This mosquito is indigenous to areas of Africa (northeast and sub-Saharan), as well as northern Asia. Females are very anthropophilic and seek huge animals, such as pigs and cattle, for blood extraction (Self *et al*. 1973, Clements, 2012, Reuben, 1994) [17, 4, 15].
Cx. Tritaeniorhynchus and Cx are two of the 27 known and suspected vectors of JE. The most significant JE vectors in India are Vishnui. The mosquito-borne virus that causes Japanese encephalitis (JE) was first identified in India in 1955. Since then, numerous significant outbreaks from various regions of the nation have been documented, mostly in rural areas. The morbidity rate is estimated to be between 0.30 and 1.5 per 100,000 people, with children being the most afflicted. Up to 50% of those who recover may have neurological impairments, and the case fatality rate has varied from 10% to 60%. Although subclinical infections have happened equally in both sexes, reported incidence has traditionally been higher in males than in females. A large number of subclinical infections occur each year during the transmission season. Diagnosis at the primary health center (PHC) level is based on clinical symptoms only. Therefore, there is a need to develop simple tests for use at the peripheral level, both for diagnosis and for epidemiological surveys.
Japanese encephalitis (JE) has been prevalent in India since the mid-1950s. It was from Tamil Nadu that the JE virus was isolated for the first time. It is essentially an animal virus, and domestic pigs are its major reservoirs. Pigs, in turn, get the infection from birds, especially pond herons and egrets, through mosquitoes. Man is just an accidental link in the disease cycle and is not a source of infection for further transmission. Cases of JE were recorded on a large scale in Uttar Pradesh, Bihar, and Assam. An extensive spread of JE was reported in Karnataka and Andhra Pradesh.
Out of 27 proven and suspected vectors of JE, *Cx. tritaeniorhynchus* and *Cx. Vishnui* are the most important vectors of JE in India. *Cx. epidesmus, An. Hyrcanus, An. subpictus, An. Barbirostris* and *Mansonia annulifera* are also known as vectors of JE in India.

JE virus has also been isolated from *Cx. Whitmorei*, *Cx. pseudovishnui, and Cx. Gelidus, Cx. bitaeniorhynchus,* and *Cx. fuscocephala.* Most of the region of Gujarat is free from JE, but it is prevalent in the north-eastern and southern parts of India.

2. Materials and Methods

The area of Study sites situated around the Rajkot City (22°20'3"N 70°45'56" E), in the semi-arid tropical region of Gujarat, where the climate was mostly hot and dry. During the study, I have selected seven villages surrounding Rajkot city for to investigation of the seasonally breeding and resting distribution pattern and density of *Culex tritaeniorhynchus* in outskirt and domestic shelters, and also compared to climatological factors. All seven selected villages have having network of canal irrigation drainage systems. Due to seepages and waterlogging from the canal creates an ideal habitat is created for the mosquitoes.



Source: Google Satellite image

Fig 1: Showing study sites in the rural areas around Rajkot city

The villages are Madhapar, Naranka, Sokhda, Kalipat, Metoda, Ronki, and Kankot, were selected for the assessment and have a semi-arid type of climate with three distinct seasons, *viz*. summer (March to June), monsoon (July to September), and winter (October to February). The study area also has small rivers in each village, and two major seasonal rivers one Aji and Nyari, also have four major dams being up for irrigation and domestic purposes in the study area.

The study was conducted from January 1, 2006, to December 31, 2007. The dipping method was used as described by WHO (1975) for the Mosquitoes and larva collection from the residence area (domestic area) and the surrounding of the residence area (outskirt area) in the morning between 6 am to 8 am. References Key was used for the identification of mosquitoes and this larva (Roy and Brown, 1971; Patel, 2002) [16, 12]. Breeding sites and resting sites of the mosquito were categorized based on observations during the sample collection of specimens. During the study period, every month, larvae and adult specimens were collected from each and every type of domestic and outskirt sites from the selected villages. These samples were further analyzed to know the status of larvae/adult and identify their presence/absence in the study area.

During the survey for the larval sampling, the dipping method was used to collect the larval specimens, and the collected samples were kept in standard plastic tubes and brought into the laboratory for identification and rearing. After that, the collected larvae specimens were reared in standard plastic jars that were covered with cotton cloth containing the feed mixed with yeast and biscuit powder mixture according to references (Das *et al.*, 2003) [5]. All the collected specimens were brought to the laboratory for species identification (Joshi, 2005) [9]. Morphological identification was done up to the species level with the help of standard références such as Roy and Brown (1971) [16], Patel (2002) [12]. Collected data were analyzed to calculate the Larval Density (LD), average monthly and village-wise larval and adult densities by using the formula LD = number of larvae collected/ number of dips made, while adult density was calculated as Man per Hour Density (MHD) by the formula MHD = Total number of mosquitoes collected / Total time of collection.

3. Results

Breeding and resting habitats preferences

The results of the field study showed that the breeding of *Culex tritaeniorhynchus* species was prevalent during the field survey and sample collection in the different breeding places of the study sites in the rural areas around Rajkot. During the field survey, 94 percent of the Breeding habitats of *Culex tritaeniorhynchus* were predominantly found in the surrounding residences (outskirts), and only 06 percent from the domestic area were recorded. In the domestic area, they were mainly preferred small vessels of domestic purposes use water as a breeding habitat followed by small vessels (03%), earth pots (02%), cattle shed (01%) and Larvae of this species were also recorded from kind of outskirt area waterbody predominantly like drainage (57%), stagnant waste water (19%), rivers (13%), rain pools (08%), and chekdam (00%). Resting habitats of the species were recorded from all types of houses throughout the year. Among the domestic habitats of human dwelling houses with huts (19%) were recorded, followed by the clay houses (16%) and cemented houses (12%), and also human dwelling houses with clay houses (20%), followed by cattle-huts (17%), cemented houses (16%), and (Table 1).

Table 1: *Cx. tritaeniorhynchus* larvae breeding preference (in percent) and resting habitat preference (in percent) of adult mosquito species at various habitats in the rural areas around Rajkot city during the year 2006-07

Breeding Habitats-*Culex tritaeniorhynchus*				**Resting Habitats**	
Domestic		**Outskirt**		**Human Dwellings: Houses without cattle**	***Culex tritaeniorhynchus***
Ground tanks	00%	Chekdam	00%	Cemented Houses	12%
Overhead tanks	00%	Rivers	11%	clay Houses	16%
Earth pots	02%	Rain Pools	07%	Huts	19%
Cattle shed with a home	01%	Stagnant wastewater	19%	**Mixed Dwellings Houses with Cattle**	***Culex tritaeniorhynchus***
Underground	00%	Drainage***	57%	Cemented Houses	16%
Barrels of plastic and metal	00%	Total	94%	Clay Houses	20%
Small vessels	03%			Huts	17%
Total-06%		***Surface ditches drain water			

Larval density-Outskirt area

In average larval density of *Cx. tritaeniorhynchus* was recorded highest level during the month of Sept-06 (5.13) and Sept-07 (5.79) in both years. Whereas Lowest was recorded from the month of May-06 (1.10) and May-07 (1.76). Village-wise average larval density was recorded as highest in village Madhaparmonth of Aug-06 (8.10), Sept-07 (8.20), and lowest was in village Naranka in the month of Mar-06 (0.55) and village Kankot in the month of Apr-07 (0.40) for species *Cx. tritaeniorhynchus* among all villages (Figure 2).

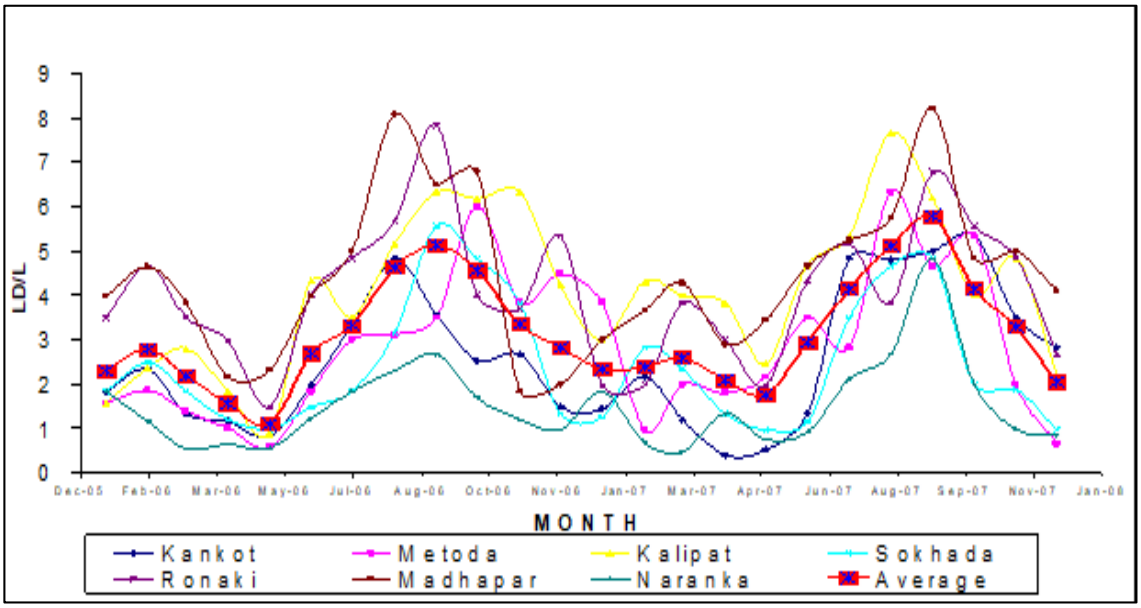


Fig 2: Month-wise outskirt Larval Density (LD) per litre of sample water of mosquito *Culex tritaeniorhynchus* of villages surveyed around Rajkot city during 2006-07(n=24/village/month)

Larval density-Domestic area

Maximum average larval density of *Cx. tritaeniorhynchus* was recorded during the month of Oct-06 (3.13) and Sept-07 (2.73), and the minimum during the month of May-06 (1.10) and Jan-07 (0.67). Village-wise average larval density was recorded as highest in village Sokhda, month of Oct-06 (4.40) and Sokhda Aug-07 (3.40) in both years. Whereas the lowest recorded was in village Sokhda, month of Apr-06 (0.50), and village Naranka, month of May-07 (0.40), of this species among all villages during field sampling (Figure 3).

Adult density (MHD)

Results of the *Cx. tritaeniorhynchus* average maximum adult density was recorded during in the month of Sept-06 (17.42) and Sept-07 (17.50) respectively both years and during this seasons in month September total rainfall was 13.40cm, 4.10cm respectively and average temperature was 28 °C and 28 °C respectively whereas minimum density was recorded during month of May-06 (7.74) and May-07 (7.0) in both years. During this month average temperature was 33 °C, and 34 °C were recorded respectively. This is the

hottest month of the season. Village-wise maximum average adult density of *Cx. tritaeniorhynchus* was recorded from village Kankot, month of Sept-06 (19.00), and village Naranka, month of Sept-07 (20.00), whereas the lowest density was recorded in Sokhad, month of May-06 (6.20), and Kalipat, May-07 (5.40), (Figure 4)..

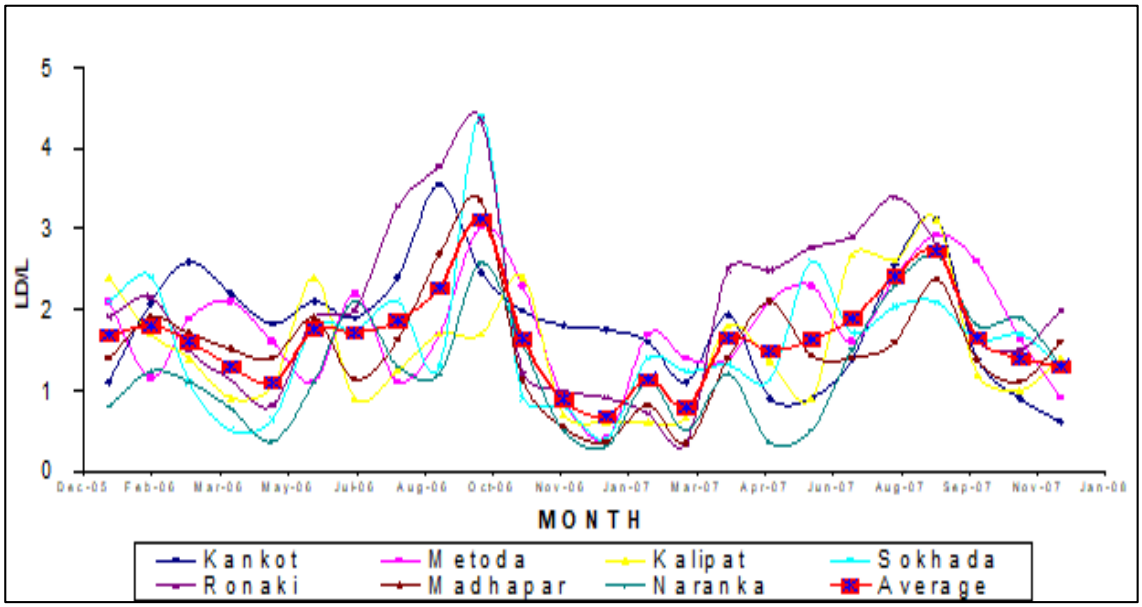


Fig 3: Month Month-wise larval density (LD) per litre of sample water stored in tanks for domestic use of mosquito *Culex tritaeniorhynchus* of villages surveyed around Rajkot city during 2006-07(n=40/village/month).

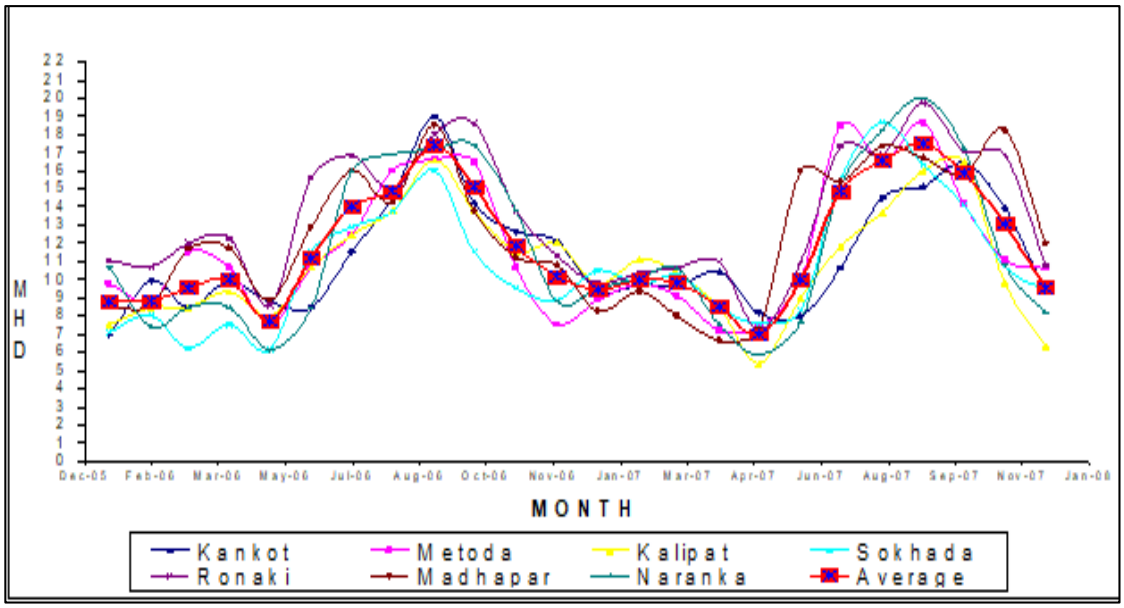


Fig 4: Month Month-wise adult MHD (Man-hour density) of *Culex tritaeniorhynchus* of villages surveyed around Rajkot city during 2006-07(n=9/village/month)

Results show that there are certain factors that have been effect on the mosquito's distribution and prevalence, like season, temperature, water table, and environmental conditions. Therefore, we have compared the average adult density with rainfall and temperature in the present study. Therefore, it was concluded from the study results that the seasonal conditions and environmental factors positively affect the adult mosquito density.

4. Discussion

The above results clearly show that the micro and macro natural and man-made temporary breeding habitats of the all-village area provide an ideal breeding platform under these conditions for the *Cx. Tritaeniorhynchus*, one of the important observations made during the study was that the open water storage tanks were built up in residences and surrounding them, and Surface open ditches of drainage water, which is ideal for the mosquitoes to breed due to low sunlight penetration and also a controlled kind of environment (Mittal *et al*, 2003) [11]. The Aquatic vegetation in larval habitats may also affect the abundance of mosquito larvae by providing protection and food, while during the present study, we have also observed that the mosquito larvae were found more in the peripheral regions having vegetation and organic matter. Many studies suggest and record that the environmental factors like rainfall and temperature, humidity, and manmade habitats are considered important factors for the breeding sites of mosquitoes and the transmission of disease. (Alicia *et al.*, 2000, Pemola and Jauhari, 2006) [1, 13].

The dependency of larval density as well as adult mosquitoes is upon the availability of breeding grounds and resting habitats in a particular area. (Yadav *et al*., 1989) [22] The higher the number of breeding and resting sites recorded, the greater the mosquito density. For example, more human-populated areas provide additional blood meal opportunities and also more breeding habitats (Batra *et al*., 1995) [2].

5. Conclusion

Mosquitoes, apart from being a biting nuisance, are responsible for transmitting diseases and creating serious public health problems, which are a burden on health services and the economic growth of nations. It is a barrier to the development of the country.

In the study areas during 2006-07 were recorded large number of breeding and resting sites of mosquitoes, Larval as well as adult species of mosquitoes, *viz. Cx. quinquefasciatus*, *univittatus*, *Cx. tritaeniorhynchus* and *Cx. Vishnui* of the genus were successfully breeding and resting, recorded in the field. Density of mosquitoes is unstable, which is mainly associated with seasonal changes in the atmospheric temperature, excessive rainfall, and availability of breeding sites and favorable resting sites. Such types of conditions were recorded in the study area (Table 1).

Keeping clean mosquito breeding ground lies on the shoulders of the residents, with the help of residents, NGO's, local institutions, and voluntary organizations, with an integrated strategy for improving conditions of the waterlogging and eradication habitats. A key challenge is to translate this efficacy into operational effectiveness through large-scale community-based programs.

During 2008, a social awareness-based study was conducted to assess awareness of people in the study area regarding one of the mosquito-borne diseases; malaria was selected for the study.

Analysis of the data shows a lack of knowledge about mosquitoes and mosquito-borne diseases, and the illiteracy level was high, which leads to a lack of awareness. Respondents knew that malaria is a mosquito-borne disease, but they had misconceptions about the malaria vector and its breeding sites.

During the study, estimating the impact of media in generating awareness, it was observed that television, friends/relatives, and newspapers are the most effective media to convey information about malaria awareness. Thus, it is suggestive that these media should be utilized to generate mass awareness in communities. It is a fact that most of the aquatic breeding habitats of mosquitoes are human-made made which is why there must be increased awareness for proper reduction of mosquito breeding grounds at all levels. Such a type of action could reduce mosquito populations by active targeting in a specific area. Socioeconomic conditions of the community have a direct bearing on the spread of diseases cannot be denied. Adequate health care and sanitation may affect the current geographic distribution and human incidence of many diseases more significantly than climate.

Awareness and education regarding mosquito-borne diseases through various programs should be carried out. Prevention of the disease through better knowledge and awareness is the appropriate way to keep the disease away and remain healthy (Yadav *et al*., 1999) [22]. People should be guided to the available facilities for the detection of various mosquito-borne diseases and the eradication of mosquitoes. Success in implementing preventive interventions amongst people is likely to be determined in part by awareness of malaria and the strategies available to prevent it and to maximize the potential for health impact and Support.

Declarations

Competing interests: The authors have no competing interests to declare that are relevant to the content of this article.

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Data Availability

All data underlying the results are available as part of the article, and no additional source data are required.

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