The WHO Regional Office for South-East Asia publish the annual Dengue Bulletin.

The objective of the Bulletin is to disseminate updated information on the current status of dengue fever/dengue haemorrhagic fever infection, changing epidemiological patterns, new attempted control strategies, clinical management, information about circulating DENV strains and all other related aspects. The Bulletin also accepts review articles, short notes, book reviews and letters to the editor on DF/DHF-related subjects. To provide information for research workers and programme managers, proceedings of national/international meetings are also published.

All manuscripts received for publication are subjected to in-house review by professional experts and are peer-reviewed by international experts in the respective disciplines.
Dengue has emerged as the most widespread and rapidly increasing vector-borne disease in the world. Of the 2.5 billion people around the world living in dengue endemic countries and at risk of contracting dengue fever, 1.3 billion live in dengue endemic areas in 10 countries of the WHO South-East Asia (SEA) Region. All Member States in the Region, except the Democratic People's Republic of Korea, being endemic to dengue, the Region contributes to more than half of the global burden of the disease. Five countries (India, Indonesia, Myanmar, Sri Lanka and Thailand) are among the 30 most highly endemic countries in the world. In spite of control efforts, there has been a significant increase in the number of dengue cases over the years, though improvement has been seen in both case management and reduction of case-fatality rate (CFR) to below 0.5%.

Compared with 2015, dengue cases in the SEA Region increased by 46% by 2019, i.e. from 451,442 cases in 2015 to 658,301 cases in 2019; and deaths decreased by 2%, i.e. from 1,584 in 2015 to 1,555 in 2019, representing a decline in the case-fatality rate (CFR) from 0.35% to 0.24%.

A variety of factors are responsible in the SEA Region for the expansion and distribution of the dengue mosquito vector and viruses, namely high rates of population growth, inadequate water, sewer and waste management systems, rise in global commerce and tourism, global warming, changes in public health policy, and the development of hyper-endemicity in urban areas, among others. The current situation of the high burden of dengue cases in the SEA Region is aggravated by the absence of effective treatment and lack of comprehensive vector control.

All these resulted in researchers engaging in studies on the clinical features, management, vector biology and control of dengue. In line with this priority, the Dengue Bulletin is published by the WHO Regional Office for South-East Asia every year, encouraging researchers to explore different aspects of the disease and contribute to the knowledge gap and evidence base for combating its rapid spread. This 41st Volume of Dengue Bulletin is in your hands. It consists of papers on the dengue burden, wolbachia, vaccines, diagnostic or therapeutic applications, early warning systems, entomological surveillance and environment management, insecticide resistance, and also the impact of the COVID-19 pandemic on dengue prevention and control efforts.

We also invite contributions for Volume 42 of Dengue Bulletin. The deadline for the receipt of the manuscripts is 31 May 2021. Contributors are requested to adhere to the instructions given at the end of this Bulletin during the preparation of their manuscripts. Contributions should either be accompanied by flash drives and sent to the Editor, Dengue Bulletin, WHO Regional Office for South-East Asia, Parsvnath Red Fort Capital Tower 1, Bhai Veer Singh Marg, New Delhi 110001, India, or by email as a file attachment to the Editor at se_denguebulletin@searo.who.int. Readers who want copies of the Dengue Bulletin may write to the same address or the WHO Country Representative in their country of residence. The pdf version will be available on the WHO Regional Office website.

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**Wolbachia-induced unidirectional cytoplasmic incompatibility in Aedes albopictus**

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**Abstract**

Wolbachia is a Gram-negative endosymbiont that harbours both mono-infection and super-infection in dengue vector, Aedes albopictus. The control of vector-borne diseases such as dengue, chikungunya, Zika, etc. rely on the phenomenon of “cytoplasmic incompatibility” (CI). CI-induced reproductive phenotype benefits infected females as they are compatible with infected or uninfected males. This study demonstrates different crosses between Wolbachia-infected and -uninfected colonies focusing on their fecundity and egg viability. In our study, a high level of CI was observed in host that are single-infected (wB) and super-infected (wAB). There was a rare egg hatch in cages with wB×w and with w×wAB showing experimental incompatibility crosses. There was no egg hatch in crosses between wB×wAB. Super-infected crosses had a higher hatch rate than that of single-infected ones. The hatch rates in compatible crosses of uninfected females and males were significantly lower than other compatible crosses. The results show that in comparison to uninfected females the infected females are at a reproductive advantage due to both CI and fitness boost associated with Wolbachia infection in terms of fecundity, egg hatch and longevity. No fitness differences were observed between single- and super-infected females. We conclude that Indian Ae. albopictus show Wolbachia-induced unidirectional CI.

**Keywords:** Wolbachia; Aedes albopictus; cytoplasmic incompatibility.

**Introduction**

The incidence of vector-borne diseases in humans can be lowered only by employing key strategies of vector control (1). Many disadvantages of the chemical control methods have prompted scientists to develop alternative control methods (2), which include genetic control strategies (3). These genetic control strategies, i.e. insect transgenic technologies, in combination with conventional approaches rely on cytoplasmic incompatibility (CI) (4).

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Consequently, there has been a considerable interest in exploring CI inducing Gram-negative endosymbiont, Wolbachia (5). Wolbachia is a maternally transmitted bacterium screened mostly in insects (6). CI is defined as non-viability of embryos that occurs as a result of mating between males carrying a definite Wolbachia strain with females that are uninfected (unidirectional CI) or are infected by a distinct Wolbachia strain (bidirectional CI). The reciprocal mating between females and males that harbour same Wolbachia infections are compatible. CI-induced reproductive phenotype benefits infected females as they are compatible with infected or uninfected males, whereas uninfected females are incompatible with infected males, thereby reducing reproductive success. Thus, Wolbachia is known to produce a saving mechanism when present in females. Males are dead-end host for maternally inherited Wolbachia. The CI offered reproductive advantage to infected females results in population replacement by spreading transgene or genetically modified strains through vector populations (7,8). CI ensures continuity of Wolbachia infection in the host population through more production of Wolbachia-infected offspring. CI is also known for target population suppression strategies with appropriate pattern of infection through the insect incompatible technique (IIT) (9). IIT has been successfully implemented against insect vectors and agricultural pests (10,11). Unidirectional CI is known to occur in both nematodes and arthropods (11–14). Studies on bidirectional CI is limited (15,16).

*Aedes albopictus*, the Asian tiger mosquito is known to be uniformly super-infected with two Wolbachia strains, viz. wAlbA and wAlbB (17). This species harbours several arboviruses afflicting humans and other animals making it an efficient vector. It originated in Asia and has invaded the tropics (18). The recent epidemic of dengue and chikungunya, transmitted by *Ae. albopictus* in India involved many human subjects (19). The abundance of breeding sites makes its control difficult with conventional methods. Several biological and ecological features of *Ae. albopictus* such as urban distribution, low dispersal, monogamy and ease of mass rearing, make it a good candidate for use in genetic control strategies (20). Being an important pest and disease vector (21), its maximum application can be found in population suppression using the Wolbachia strategy.

The current vector control methods such as larviciding, source reduction and fogging has proved to be less worthy as there is an alarming increase in the population of *Ae. albopictus* (22). These control strategies along with release of insects with dominant lethality (RIDL) and use of Wolbachia(23–25) targeted chiefly *Ae. Aegypti*. The emergence of *Ae. albopictus* as a vector can be linked with suppression of *Ae. aegypti* population. Thus it is important to study the dynamics of *Ae. albopictus* to evolve measures for its control. This biological control method uses the phenomenon “Cytoplasmic Incompatibility” to reduce host population (11,26,27). *Ae. albopictus*, a known vector of dengue and chikungunya, harbours native Wolbachia which makes it the most suitable candidate to study CI. This study aims to know the status of Wolbachia-induced CI in *Ae. albopictus* population of India. This can be shown with different crosses between Wolbachia-infected and uninfected colonies focusing on their fecundity and egg viability. The study may help to control *Ae. albopictus* population in India.
**Materials and methods**

*Ae. albopictus* strains and colony maintenance/experimental study

The *Wolbachia* super-infected and single-infected colonies of *Ae. albopictus* were established in the insectariums. These colonies were taken from Khordha and Jajpur districts of Odisha, respectively. The non-infected colony was generated from Khordha samples by feeding newly emerged adults with sucrose solution supplemented with tetracycline and B-complex (28) followed by rearing of their offspring in tetracycline (29). Two subsequent generations after tetracycline treatment to adults and larvae were free from the antibiotic treatment for the restoration of normal microbiota in them and also to recover from any perceived side-effects caused during tetracycline treatment. The fourth generation of infected offspring was used for the experiment. The emerged adults were fed on 10% sucrose solution. Mosquito colony maintenance was done at 28±2 ºC temperature, 75±5% relative humidity (RH) and 12 dark: 12 light photoperiod. Thus, the experimental study included three *Ae. albopictus* strains: Wolbachia super-infected (*w*<sup>Ab</sup>) males and females; *Wolbachia* single-infected (*w*<sup>B</sup>) males and females and *Wolbachia* cleared (*w*<sup>-</sup>) males and females. The females and males from each strain were maintained in separate cages for crossing experiments.

Experimental cages for mating

The experimental crosses consisted of nine different cages with four replicates of each (Fig. 1). Each of the cages had 30 virgin females of same age that survived only on 10% sucrose solution. Thirty virgin/newly emerged males (2–5 days old) were placed into the cages for mating for 2 days when the females were 5 days old. Subsequently, rabbit blood meal was provided to the females for egg laying. A record on daily collection of eggs was maintained. The eggs were dried and hatched to measure their viability. The larvae from each colony were provided with yeast powder and dog biscuit and reared in enamel trays. A record of surviving adult males and females was maintained. The oviposition filter papers were collected after the death of the last female. The filter papers with ≥100 eggs were taken for measuring the CI levels (30).

PCR amplification

Screening for *Wolbachia* in *Ae. albopictus* was confirmed by strain-specific primers from the *wsp* gene (*wspFN/wAlbAN for wAlBA and *wspFN/wAlbBN for wAlB*) (31). The reaction mixture for PCR included 0.25 mM dNTPs, 2.5 mM MgCl<sub>2</sub>, 1.8 mM primers and 1 U Taq DNA polymerase and 100 ng DNA following cycling conditions: 95 ºC for 5 min, followed by 35 cycles of 95 ºC for 1 min, 56 ºC for 1 min and 72 ºC for 2 min, followed by 72 ºC for 10 min; 10 µl of the PCR products were run 1.5% agarose gel.
Fig. 1. Schematic representation of experimental crosses in each cage (Wolbachia super-infected: w^{AB}; Wolbachia single-infected: w^{B}; Wolbachia uninfected: w). All the crosses mentioned are female x male (cross marks in the figure indicate unviable offspring).

Statistical analysis

All the calculations were performed using the statistical package of Microsoft XLSTAT 2007. Single-way ANOVA ($P<0.001$) was used to assess the statistical difference between compatible and incompatible crosses.

Results

Before the experiment, the infection status was determined through PCR. The *Ae. albopictus* samples from Khordha and Jajpur were found to be super-infected (w^{AlbA} and w^{AlbB}) and single-infected (w^{AlbB}), respectively. The samples from Khordha that were subjected to tetracycline curing were devoid of both single-and super-infection.
**Wolbachia infection status of offspring from experimental crosses**

As evident from PCR analysis during the experiment, the offspring from \( w^-x^- \) were not infected with *Wolbachia*. The offspring from \( w^Bx^B \), \( w^Bx^A^B \) and \( w^Bx^A^A^B \) were uninfected. The offspring from \( w^Bx^- \) and \( w^Bx^B \) were single-infected with \( w^A^B^B \). The offspring from \( w^A^B^A^- \), \( w^A^B^A^B \) and \( w^A^B^-x^B^B \) were super-infected.

**Fecundity**

The mean number of eggs produced in super-infected cross \( w^A^B^Bx^A^B \) (10 194.5) was larger than the cross \( w^A^B^Bx^- \) (10 056.6) and cross \( w^A^B^Bx^B(5306.6) \). The mean number of eggs produced in single-infected cross \( w^Bx^B \) (9775.3) was larger than \( w^Bx^- \) (6745.8). The mean number of eggs produced in uninfected cross \( w^-x^- \) was 6471.8. The mean number of eggs in the incompatible crosses \( w^-x^B \), \( w^-x^A^- \) and \( w^-x^A^B \) were 2159.6, 2214.3 and 2146.7, respectively (Fig. 2).

*Fig. 2.* Bar graph of mean number of eggs laid by each female of each cross

**Egg viability**

The mean highest egg viability percentage was observed in single-infected cross \( w^Bx^B \) (87.4) followed by super-infected cross \( w^A^B^Bx^A^B \) (85.9). The mean egg viability percentage in uninfected cross \( w^-x^- \) was 64.6. The mean egg viability percentage in single- \( (w^B^-x^-) \) and super-infected crosses \( (w^A^B^-x^-) \) and \( w^A^B^-x^B \) were 83.9, 82.8 and 79.3, respectively. The mean egg viability percentage in incompatible crosses \( w^-x^B \), \( w^-x^A^B \) and \( w^-x^A^A^B \) were 0.07, 0.04 and 0.00, respectively (Fig. 3).
Longevity

Survivorship of both males and females was similar for infected strains whereas it was lower for uninfected strains. The single- and super-infected adults lived longer than uninfected counterparts. The uninfected females lived up to 24 days and males up to 21 days. Both single- and super-infected adults lived up to 33 days (females) and 27 days (males) (Fig. 4).

Statistical analyses

The fecundity was significantly different in compatible and incompatible crosses. The fecundity was higher in cross where male and female had super-infection. The compatible crosses where either of females and males had single-infection or super-infection had a significant difference in fecundity and a non-significant difference in egg hatching percentage at \( P < 0.05 \). The compatible crosses between \( w_B^B/w_A^B \) and \( w \) had significant difference in fecundity and a non-significant difference in egg hatching percentage at \( P < 0.05 \). There was a higher hatch rate when mating occurred between \( w_A^B/x^w \) compared to \( w_A^B/x^w_B \). The compatible crosses between \( w_A^B \) and \( w/W_B^B \) had a significant difference in fecundity. The compatible crosses of \( w/x^w \) had a lower egg hatch compared to \( w_B^B/x^w_B \) and \( w_A^B/x^w_A^B \). All the incompatible crosses had no significant difference in fecundity and egg hatching percentage at \( P < 0.05 \).

Discussion

CI is widespread in nature in forms of pollen sterility in grains, maternally inherited sex ratio and male-sterility factors in Drosophila and cytoplasmically determined infertility in mosquitoes (32). The cause of CI in mosquitoes can be attributed to the presence
Wolbachia-induced unidirectional cytoplasmic incompatibility in Aedes albopictus

**Fig. 4.** Longevity of adult (A) females and (B) males

![Graph A: Longevity of adult females](image)

![Graph B: Longevity of adult males](image)

of endosymbiont Wolbachia within germ cells (33,34). CI has been exploited widely in Drosophila sp., Cx. pipiens and Ae. albopictus. Cytological investigations for understanding the mechanism of CI has been done on D. simulans (35). Wolbachia-induced CI is defined as failure to complete embryonic development in eggs laid following crosses between uninfected female and infected (single- or super-infected) male or female and male infected with different Wolbachia strains. This renders crosses between different populations to be fertile, sterile in one or both directions. Owing to this phenomenon, infected females get fitness advantage over uninfected females producing viable offspring thereby replacing uninfected
unidirectional cytoplasmic incompatibility in Aedes albopictus

ones in any mixed population, showing an ideal or perfect maternal transmission. CI is unidirectional when a super-infected female is fully compatible with single-infected males, thereby allowing super-infected population to spread in a mixed population (36). CI also reduces development of Wolbachia-free offspring.

Ae. albopictus populations are naturally super-infected or single-infected with Wolbachia (31). In a cross between super-infected male and super-infected female in Ae. albopictus, the sperm is modified by two independent molecules which can be rescued only by oocytes containing rescue molecules for the both. This produces viable eggs. In a cross between single-infected female and super-infected male, oocytes can rescue only one modification of the super-infected sperms resulting in unviable eggs. A cross between uninfected female and single- or super-infected male gives sterile eggs. A sterile cross occurs when single-infected female mates with super-infected male. In crosses between super-infected females and super-infected or single-infected males, oocytes can rescue the sperm of single or super-infected males producing viable eggs (13). This explains unidirectional CI in Ae. albopictus. A cross between uninfected female and single-infected male results in developmental arrest and production of sterile eggs whereas fertile eggs are produced in cross between single-infected female and uninfected male.

The CI in Ae. albopictus (single- or super-infected) can be attributed to modification and rescue. In our study, a high level of CI was observed in host that are single-infected (wB) and super-infected (wAB). There was rare egg hatch in three of our experimental incompatibility crosses. This shows that there was a normal insemination, penetration of sperm to eggs followed by embryonic development and organ formation in form of high percentage of eggs from which only few hatched. In our study, rare egg hatch was found in cages with w×wB (0.07%) and cages of w×wAB (0.04%). A cross between w×wB has a higher egg hatch than w×wAB. This can be due to infrequent failure of the modification mechanism of Wolbachia. There was no egg hatch in crosses between wB×wAB. These results on egg hatch are different from the previous studies where there were no egg hatches between incompatible crosses (12,29). Earlier studies reported a mean egg hatch of 0.19–0.40% when super-infected male from two populations (CH and KL) mated with female UJU (uninfected) or single-infected (KOH) showing a high degree of CI. This is in contrast to mean egg hatch of 88.6–88.9%, which was observed when super-infected wild male mated with super-infected (KLPP) laboratory female (37). Other studies reported that Australian D. simulans with natural Wolbachia did not exhibit CI (38) while Wolbachia caused bidirectional CI in D. simulans (15). The same host can have different types of Wolbachia-induced CI. Dobson et al. (14) reported Wolbachia-induced unidirectional CI in Ae. albopictus with absolute embryonic death. This suggested that spread of Wolbachia infection is due to a combination of both CI and augmented host fitness.

The hatch rates in compatible crosses significantly differed from one another. Super-infected cross had a higher hatch rate than that of single-infected ones. This may be due to fitness advantage offered by Wolbachia and genetic variations of the host (30). Super-infected
female when mated with uninfected male had a hatch rate of 82.8%. The hatch rate of 79.3% between super-infected female and single-infected male may be due to infrequent failure of the rescue mechanism. This is a failure of maternal transmission as reported in studies by Subbarao et al. (39) and Turelli and Hoffmann (40). The hatch rates in compatible crosses of uninfected females and males were significantly lower than other compatible crosses. This decreased hatch rate may be due to curing of Wolbachia, further confirming that Wolbachia gives a reproductive advantage to Ae. albopictus (41). To confirm that Wolbachia was the sole contributing factor affecting fecundity and survival, Wolbachia-uninfected colonies were reared without tetracycline for two generations for restoring lost microbiota (42).

In a previous study, we reported 80% of Ae. albopictus to be super-infected with Wolbachia (31). A cross between Ae. albopictus male with low density of wAlbA and Ae. albopictus female transfected with wPip resulted in complete CI in contrast to crosses between males with high density of wAlBA and wPip female (43). This may be due to a low density of wAlBA than wAlbB in naturally infected Ae. albopictus (31). In this study, however, 99.3% and 99.6% embryonic death from crosses between uninfected female and single- or super-infected male may be due to the varying density of wAlbA in samples.

In our study, Wolbachia infection affected female fecundity and longevity. Super-infected females had more eggs laid than single-infected ones. Both single and super-infected males and females lived longer than uninfected counterparts. This suggests non-virulence of Wolbachia infection in both males and females. In contrast, Dobson et al. (30) reported uninfected males were longest lived.

Several studies have shown the effect of male age on CI. A high CI was observed in crosses between virgin (2–5 days old) single-infected (KOH) male mated with uninfected (UJU) female. The level of CI further declined with an increase in age of male, i.e. 10-days old male (18.3% egg hatch) and 60 days old male (43.6% egg hatch). In contrast, a high degree of CI was observed when virgin super-infected (KLPP) males from different age classes (2–5 days to 60 days old) mated with young females (1–3 days old) that were uninfected (UJU) or single-infected (KOH) (37). In our study, the high level of CI may be due to mating between single- or super-infected newly emerged virgin males (2–5 days old) with uninfected females.

The results show that compared to uninfected females the infected females are at a reproductive advantage due to both CI and fitness boost associated with Wolbachia infection in terms of fecundity, egg hatch and longevity. No fitness differences were observed in comparison of single- and super-infected females. A reduction in Ae. albopictus population can occur with continuous field release of uninfected male and female that will produce unviable eggs on mating and reduce adult longevity. The released uninfected male can also mate with naturally infected female producing unviable offspring and reducing Ae. albopictus population. This will help to study the effects of CI. Further studies on the effect of both native and transfected Wolbachia in Ae. albopictus need to be done. The nativity of super-infection and single-infection of Wolbachia is responsible for their invasion and maintenance within the
host *Ae. albopictus*. Sinkins et al. (44) reported that super-infection invaded a single-infected population. Likewise, in our experiment the single-infection B might have been invading the uninfected population inducing high levels of CI.

This study is based on laboratory crosses highlighting in-depth knowledge on CI, fecundity and longevity of *Ae. albopictus* adults. Further compatibility tests can be done using field collected males or via introgression that may depict a variation in host fitness. Based on the these results, we can conclude that Indian *Ae. albopictus* show Wolbachia-induced unidirectional CI.

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**Conflict of interest**

The authors declare no conflicts of interest.

**Ethical approval**

All procedures performed in the study involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

**References**


Wolbachia-induced unidirectional cytoplasmic incompatibility in Aedes albopictus


Wolbachia-induced unidirectional cytoplasmic incompatibility in Aedes albopictus


Dengue vaccine acceptance among international students in a USA university

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Abstract

This study surveyed international undergraduate students of at least 18 years of age from 17 countries and one territory who attended a USA university on their awareness of dengue fever, possible acceptance of a dengue vaccine, and rationale for those not desiring to obtain a dengue vaccine. The survey was conducted through the QualtricsXM online platform. The majority of the students planned not to receive a dengue vaccine in the future. Rationale for avoiding the dengue vaccine included fear of vaccination, side-effects and costs. Variations of dengue vaccine receptivity were observed between students from different regions. Students from Africa were less likely to desire to receive a future dengue vaccine compared to Asia-Pacific and Latin America/Caribbean regions.

Keywords: Vaccine acceptance; international students, Latin America

Introduction

Dengue fever continues to be a major world health problem (1). The advent of dengue vaccines brings possibilities of improved dengue control to lower dengue morbidity and mortality (2). Besides the licensed CYD-TDV vaccine, also known as Dengvaxia®, five other dengue vaccines are in the development stage (3). As of 2019, clinical trials for CYD-TDV occurred in a limited number of 20 Asia-Pacific and Latin American countries. Vaccine efficacy appeared to be associated with the age of recipient (4).

As the dengue vaccine begins to roll out across the world, social awareness and support become essential components to promote dengue vaccine adoption in populations (5). Attitudes towards dengue vaccine acceptance vary around the world. For example, highly favourable attitudes towards possible dengue vaccine usage reached the 80% to 90% favourability in community studies conducted in Colombia (6,7), Venezuela (7), Vietnam (8) and Malaysia (9). A study from Indonesia yielded dengue vaccine favourably in the upper 70% favourability (10). In contrast, dengue vaccine acceptance in Singapore for the possible use of the dengue vaccine amounted to only 21% of those surveyed (11).

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The rationale for dengue vaccine acceptance varied from study to study. Dengue vaccines acceptance correlated per study. Dengue vaccine acceptance correlated with finances in several studies (6–10). Also, educational (6, 10) level an age-related to dengue acceptance (10). A review study on factors associated with dengue vaccine acceptance stated, “Attitude toward vaccination and SES are associated with both acceptance and WTP (willingness to pay) for DV (dengue vaccine).” That study emphasized the importance of other variables such as dengue knowledge, and dengue history. Further, the price of dengue vaccine could not be ignored when considering dengue vaccine acceptance (12).

Students provide a unique population subset as to opinions related to vaccinations. Students had low vaccination rates for vaccines such as the influenza vaccine, at 43% for a group of public health students in the USA (13), and 42% among a group of medical students in Brazil (14). Only 2% of medical and health-related students received the full series for the hepatitis B vaccine (15). Less than 1.5% of university level students in Turkey received the human papilloma virus (16). Consequently, vaccine acceptance may depend upon location as well as type of vaccine.

University students provide an important segment of the population to ascertain dengue vaccine acceptance. They are future leaders and influencers. Their opinions about dengue vaccines yield important information to develop future programmes, especially as dengue vaccines become available in new countries. This study seeks to explore the attitudes and beliefs of international students in a university population in the USA.

**Materials and methods**

**Participants**

The Institutional Review Board (IRB) of a Virginia, USA university first approved this study with IRB approval number: 3971.110719. Selection criteria for participants included registration as a residential student, at least 18 years old, holder of a non-resident educational visa and classification as an international student from a home country with the presence of dengue fever. Citizens or nationals from the USA states or territories were excluded from this study. Two hundred sixty-five students from 45 nations were identified for possible inclusion for the study. International students from countries without dengue fever cases were excluded. Participation was voluntary. This sample of international students was obtained in a USA university located outside of the “dengue zone”. This allowed the international student population to be free of local media promotion and social support to consider a future dengue vaccination.
Questionnaire

A seven-item questionnaire related to dengue vaccine awareness and acceptance was developed by two public health professionals and included an external public health professional review for content and flow. Three questionnaire items addressed demographics, while four addressed issues related to dengue. The questionnaire was delivered through the QualtricsXM online platform (17).

Procedure

The administration of the university processed a list of email addresses of qualified potential international student candidates. No names of the students were given to the researchers to maintain confidentiality. If potential student candidates had interest in the study, they would respond to complete the informed consent form on QualtricsXM (17). Once agreed, they continued to fill out the questionnaire. No student names or other identifiers were available for the researchers. Statistical analyses of percentages and Chi-square test of independence were accomplished through QualtricsXM (17). The questionnaire was available from 15 November to 8 December 2019.

Results

Twenty-seven eligible students from 17 countries and one territory enrolled in the study. The countries were divided among the following regions: Asia-Pacific, Africa, Latin America/Caribbean, and not identified. The students came from eight academic areas of study. There was no majority academic area of study. The majority of the students were females (Table 1).

About two thirds of the students had an awareness of dengue fever (Table 2). Only a minority of the students would receive the dengue vaccine (Table 3). For those who do not wish to receive the dengue vaccine, fear of vaccines was the greatest reason to avoid the dengue vaccine (Table 4). Among those uncertain to receive the dengue vaccine in the future, the majority responded to the “other” category (Table 5).

Cross-tab analysis to examine dengue fever awareness by geographical region revealed a statistically significant difference, Chi-square=39.174, $P<0.001$. The Asia-Pacific region yielded the least dengue fever awareness, followed by the African region (Table 6). Cross-tab analysis to examine dengue vaccine receptivity by geographical region also revealed a statistically significant difference, Chi-square=115.880, $P<0.001$. The Africa region yielded the least support for dengue vaccine receptivity, followed by the Asia-Pacific region (Table 7).
Table 1. Demographic data

<table>
<thead>
<tr>
<th>Demographic</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>22.2</td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
<td>70.4</td>
</tr>
<tr>
<td>Not identified</td>
<td>2</td>
<td>7.4</td>
</tr>
<tr>
<td>Region of country of origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asia-Pacific</td>
<td>13</td>
<td>48.1</td>
</tr>
<tr>
<td>Africa</td>
<td>6</td>
<td>22.2</td>
</tr>
<tr>
<td>Latin America/Caribbean</td>
<td>4</td>
<td>14.8</td>
</tr>
<tr>
<td>Not identified</td>
<td>4</td>
<td>14.8</td>
</tr>
<tr>
<td>Area of studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health sciences</td>
<td>10</td>
<td>37.0</td>
</tr>
<tr>
<td>Business</td>
<td>5</td>
<td>18.5</td>
</tr>
<tr>
<td>Engineering</td>
<td>4</td>
<td>14.8</td>
</tr>
<tr>
<td>Others</td>
<td>6</td>
<td>22.2</td>
</tr>
<tr>
<td>Not identified</td>
<td>2</td>
<td>7.4</td>
</tr>
</tbody>
</table>

Table 2. Dengue fever awareness

Q. Are you aware of the disease known as dengue fever?

<table>
<thead>
<tr>
<th>Answer</th>
<th>%</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>68.00</td>
<td>17</td>
</tr>
<tr>
<td>No</td>
<td>12.00</td>
<td>3</td>
</tr>
<tr>
<td>Not sure</td>
<td>20.00</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 3. Dengue vaccine receptivity

Q. Would you receive the dengue vaccine?

<table>
<thead>
<tr>
<th>Answer</th>
<th>%</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>40.74</td>
<td>11</td>
</tr>
<tr>
<td>No</td>
<td>14.81</td>
<td>4</td>
</tr>
<tr>
<td>Not sure</td>
<td>44.44</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>27</td>
</tr>
</tbody>
</table>
Table 4. Rationale for those not receiving the dengue vaccine

Q. Why would you not receive the dengue vaccine?

<table>
<thead>
<tr>
<th>Answer</th>
<th>%</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>I am afraid of vaccines</td>
<td>50.00</td>
<td>2</td>
</tr>
<tr>
<td>It may cost too much</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>I already had the dengue vaccine</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>I believe the vaccine will actually make it easier for me to come down with dengue</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>I believe it may cause side-effects</td>
<td>25.00</td>
<td>0</td>
</tr>
<tr>
<td>It is against my religious beliefs</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>25.00</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
<td><strong>4</strong></td>
</tr>
</tbody>
</table>

Table 5. Rationale for those who are uncertain to receive the dengue vaccine

Q. Why are you not sure whether you will receive the dengue vaccine?

<table>
<thead>
<tr>
<th>Answer</th>
<th>%</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>I am afraid of vaccines</td>
<td>14.29</td>
<td>2</td>
</tr>
<tr>
<td>It may cost too much</td>
<td>7.14</td>
<td>1</td>
</tr>
<tr>
<td>I already had the dengue vaccine</td>
<td>7.14</td>
<td>1</td>
</tr>
<tr>
<td>I believe the vaccine will actually make it easier for me to come down with dengue</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>I believe it may cause side-effects</td>
<td>14.29</td>
<td>2</td>
</tr>
<tr>
<td>It is against my religious beliefs</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>57.14</td>
<td>8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
<td><strong>4</strong></td>
</tr>
</tbody>
</table>

Table 6. Dengue fever awareness by region

Q. Are you aware of the disease known as dengue fever?

<table>
<thead>
<tr>
<th>Region</th>
<th>No (%)</th>
<th>Not sure (%)</th>
<th>Yes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latin America/Caribbean</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Africa</td>
<td>33.3</td>
<td>40</td>
<td>25</td>
</tr>
<tr>
<td>Asia-Pacific</td>
<td>66.6</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>99.9</strong></td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Pearson Chi-square ($\chi^2$)=39.174, $P<0.001$; df=2
Table 7. Dengue vaccine receptivity by region

**Q. Would you receive the dengue vaccine?**

<table>
<thead>
<tr>
<th>Region</th>
<th>No (%)</th>
<th>Not sure (%)</th>
<th>Yes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latin America/Caribbean</td>
<td>0</td>
<td>8.3</td>
<td>37.5</td>
</tr>
<tr>
<td>Africa</td>
<td>75</td>
<td>16.7</td>
<td>25</td>
</tr>
<tr>
<td>Asia-Pacific</td>
<td>25</td>
<td>74.7</td>
<td>37.5</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>99.7</td>
<td>100</td>
</tr>
</tbody>
</table>

Pearson Chi-square ($\chi^2$)=115.880, $P<0.001$; df=2

**Discussion**

The sample included participants from across the globe, from various regions, and countries where dengue infection is present. While the participants held no majority academic discipline, health sciences, encompassed the greatest number of participants. The majority of participants were females (Table 1) since the majority of this university’s student population were females.

Over two thirds of the participants expressed awareness of dengue fever (Table 2), yet only a majority (40.74%) would permit the administration of the dengue vaccine (Table 3). This acceptance level is similar to the students’ acceptance of influenza vaccine in the USA at 40+% acceptance rate (13). The results of this study contrasted the results of dengue vaccine acceptance rates in other studies (6–10).

Fear of vaccination was the first reason for not desiring to accept the dengue vaccine (Table 4), and tied for the second leading response for those participants who were uncertain whether to receive a dengue vaccine (Table 5). The concern about side-effects concerned both those participants that would not receive the dengue vaccine (Table 4), as well as those who were uncertain to receive the dengue vaccine (Table 5). The rationale of dengue vaccine side-effects also concerned parents in the Philippines (18). Religious beliefs were not a rationale for those participants not planning to receive the dengue vaccine (Table 4), as well as those participants uncertain to receive the dengue vaccine (Table 5). Only one participant in the entire study indicated their previous dengue vaccination caused uncertainty to receive a dengue vaccine again (Table 5). The CYD-TDV vaccine has a three-dose schedule (3). Vaccine recipients, therefore, need to be aware of possible booster doses. Only one participant in the study indicated vaccine cost as a possible reason for not receiving the dengue vaccine (Table 5). This response may be related to the financial capacity of the international students. Both participants’ responses to not receiving the dengue vaccine (Table 4), and uncertainty to receive the dengue vaccine (Table 5) yielded the response of “other”. The greatest response for uncertainty to receive the dengue vaccine was “other” (Table 5).
The lower fever awareness among participants from the Africa region (Table 6) may be driven by the current lower burden of dengue in Africa (1). The Asia-Pacific region however, had both high “yes” and “no” responses to dengue awareness (Table 6). There responses may reflect the variation of dengue prevalence in that region. Dengue vaccine receptivity ranked the lowest in the Africa region (Table 7). The Asia-Pacific region ranked the lowest for uncertainty to receive the dengue vaccine (Table 7). These results appear in contrast to Indonesia study where dengue vaccine favourability related to educational level (10).

Limitations of the study included its small sample size, and sample make-up. While this study had a small sample size, it should be noted that this is a preliminary study. The study sample excluded graduate students and also the online student population. The study relied on only self-reports. Lack of open-ended responses further limited the breadth of possible responses for not accepting dengue vaccination.

**Conclusion**

The majority of the students either planned not be vaccinated or had uncertainties concerning a future dengue vaccination. Various reasons for avoiding dengue vaccination include fear of vaccinations, side-effects, and to a lesser extent, costs of vaccination. The predominant response choice was “other” for students unsure of receiving a dengue vaccine. Dengue fever awareness, and dengue vaccine receptivity varied by region of participants with participants from the Africa region least likely to receive a dengue vaccine. Future research needs to address not only these stated concerns for vaccine avoidance, but also elicit those other unknown concerns related to vaccination. Focus groups and open-ended response studies help in determining unknown concerns of these students.

**References**


Dengue vaccine acceptance among international students in a USA university


Dengue burden

### Burden of dengue in northern India

**A. Biswas, a# P. Sohal, a P. Singla, a P. Coshic, c A.C. Dhariwal, b N. Dhingra, b K. Baruah b**

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*National Vector Borne Disease Control Programme, Ministry of Health and Family Welfare, Government of India*

*Department of Transfusion Medicine, AIIMS, New Delhi, India*

**Abstract**

**Background:** Dengue is a vector-borne disease; however, non-vectorial transmission of dengue has been completely overlooked. A majority of dengue infection is known to be asymptomatic; however, during acute subclinical stage one might be infectious. One could also transmit the virus through blood transfusion at the time of acute asymptomatic stage during the early 5 days of viraemia. We planned to estimate the burden of dengue in the capital region of India.

**Methodology:** Seropositivity of dengue for IgM Ab, NS1 Ag and IgG Ab were performed among the samples of blood donors to estimate the seroprevalence over 3 years.

**Results:** A total of 1558 healthy blood donors were screened for the study. On the basis of inclusion/exclusion criteria, we enrolled 1531 subjects for the study. Twenty-seven donors were excluded, of which six were detected HIV-positive, 11 were positive for HBsAg and 10 were found positive for HCV. Their mean age was 30.51±7.75 years. Of 1531 subjects, 18 (1.18%) had a past history of typhoid fever, 28 (1.83%) of chikungunya fever, 9 (0.59%) of malaria and 43 subjects (2.81%) of symptomatic dengue infection. Regarding NS1 Ag seropositivity, 2.22% (34) of subjects were found positive for NS1 Ag with a peak point prevalence of 7.14% in October 2018. IgM Ab was tested and seropositivity was detected in 5.49% (84) of subjects with a peak point prevalence of 14.29% in October 2018. IgG seropositivity was detected in 64.21% (983) of subjects.

**Conclusion:** Blood samples in blood banks should be tested for dengue before transfusion to prevent transfusion transmitted dengue infection as we estimated 2.22% positivity of NS1 Ag in our study.

**Keywords:** Dengue seroprevalence; asymptomatic dengue

**Introduction**

Dengue is a vector-borne disease; however, dengue virus transmission without mosquito vector has been reported to occur via different routes such as blood transfusion, needle-stick injury, intrapartum, bone marrow transplant and mucocutaneous exposure. One could
transmit the virus through blood transfusion at the time of acute asymptomatic stage during the early 5 days of viraemia. Transfusion-related dengue transmission could cause a serious problem especially in areas highly endemic for dengue.

Methods

This cross-sectional study was conducted in All India Institute of Medical Sciences (AIIMS), New Delhi to estimate the burden of dengue infection among blood donors who are residents of Delhi and the National Capital Region (NCR). Blood samples were collected for testing NS1 antigen, IgM and IgG antibody from the subjects to look for acute as well as past dengue infection.

Observations and results

A total of 1558 donors were screened for the study. On the basis of inclusion/exclusion criteria, we enrolled 1531 donors for the study. Twenty-seven donors were excluded, of which 6 were detected HIV-positive, 11 were positive for HBsAg and 10 were found positive for HCV. Their mean age of subjects was 30.51±7.75 years. Around 56.7% (868) of subjects were in the age group less than or equal to 30 years, around 38.5% (589) were in the age group of 31–45 years, while only 4.8% (74) were more than 45 years of age. Most of the subjects were males (98.6%), implying that a majority of donors are males. Around 76.2% (1166) of subjects belonged to Delhi while around 23.8% (365) belonged to the NCR. Of 1531 subjects, 18 (1.18%) had a past history of typhoid fever, 28 (1.83%) of chikungunya fever, 9 (0.59%) of malaria and 43 subjects (2.81%) of symptomatic dengue infection. Around 2.22% (34) of subjects were found positive for NS1 Ag, seropositivity of IgM Ab was 5.49% (84) of subjects and 64.21% (983) of subjects were found positive for IgG Ab.

Of 1166 subjects who are residents of Delhi, 2.06% (24) of subjects were NS1 Ag-positive, about 5.83% (68) of subjects were IgM Ab-positive and about 64.15% (748) of subjects were IgG Ab-positive, while out of 365 residents of NCR, 2.74% (10) subjects were positive for NS1 Ag, 4.38% (16) of subjects were positive for IgM Ab and 64.38% (235) of subjects were positive for IgG Ab. We calculated the seropositivity of NS1 Ag month-wise over the study period. This way we could estimate the point prevalence of asymptomatic dengue infection, which can possibly be actively transmitted through blood transfusion. We observed NS1 Ag seropositivity mainly during the outbreak season (July till November) with peak point prevalence in October 2018 (7.14%) (Fig. 1).
We compared the positivity of NS1 Ag among residents of Delhi and NCR (Fig. 2). We calculated month-wise seropositivity of IgM Ab among the donors over the study period (Fig. 3). The peak point prevalence was observed in October 2018 (14.29%). Month-wise percentage positivity of IgM Ab among residents of Delhi and NCR was compared (Fig. 4). We calculated month-wise seropositivity of IgG Ab among the blood donors during the study period (Fig. 5).
Figure 3. Point prevalence (month-wise positivity) of IgM Ab in percentage

Figure 4. Comparison of month-wise percent positivity of IgM Ab among residents of Delhi and NCR
Dengue burden

Figure 5. Showing month-wise positivity of IgG Ab in percentage

Dengue infection prevalence

(NS1 only + IgM only + IgG only + NS1 & IgM + NS1 & IgG + IgM & IgG) The peak point prevalence of dengue infection was found to be 83.33% in September 2018 during the study period (Fig. 6). The 3-year period prevalence of dengue infection was found to be 67.41% (1032) as shown in Table 1.

Figure 6. Point prevalence of dengue infection (NS1 only + IgM only + IgG only + NS1&IgM + NS1&IgG + IgM&IgG) during the study period
### Discussion

We found 2.22% of subjects (34) positive for NS1 Ag. There have been studies on estimation of NS1 Ag seropositivity among healthy subjects. Ahmed et al. estimated the seroprevalence of asymptomatic dengue infection and its antibodies among Saudi blood donors. They collected blood samples from 910 healthy adult male donors between March 2015 and August 2016 and found 5.3% seropositivity for NS1 Ag (1). In a pilot study, Ahmed et al. studied the seroprevalence of dengue infection among blood donors in the western region of Saudi Arabia. Blood samples of 100 healthy male donors were collected for testing NS1 Ag, IgM Ab and IgG Ab; seropositivity for NS1 Ag was found to be 1% (2). Non-vectorial transmission of dengue infection has also been reported. Nemes et al. reported nosocomial transmission of dengue in a physician, who while collecting a blood sample from dengue-infected patient, accidentally struck her finger with the needle, later developed fever, headache, diffuse maculopapular rash, myalgia, cervical lymphadenopathy and malaise. In the serum sample obtained from her 12 days after onset, IgM antibodies to dengue viruses were found. Both IgM and IgG antibodies were found in serum samples from this patient 3 weeks after onset of her illness (3). Chen et al. reported a case of nosocomial dengue infection that was transmitted by mucocutaneous exposure to blood from a febrile traveler who had recently returned from Peru. Both the traveller and the health-care worker were subsequently found to have dengue fever with dengue virus type 3 (4). Tambyah et al. reported dengue hemorrhagic fever transmitted by blood transfusion. A 52-year-old, asymptomatic, repeat blood donor developed fever the day after donation. He was positive for dengue virus type 2, as ascertained by polymerase chain reaction (PCR) assay. The recipient of the donor’s red cells had fever and myalgia 2 days after transfusion. The recipient of the donor’s fresh-frozen plasma had fever and worsening pleural effusions the day after transfusion. Both recipients were positive for dengue virus type 2 (5). Wagner et al. reported a case of nosocomial transmission of dengue virus in Germany by a needle-stick injury. Diagnosis was confirmed by seroconversion and detection of dengue viral RNA by Taq Man RT-PCR (6).

Dengue virus-specific IgM antibodies are usually detected from day 5 of illness and can persist for 30–60 days. We found 5.49% (84) seropositivity for IgM Ab. There have been studies on seroprevalence of IgM Ab. Heffelfinger SB et al. on the basis of their sero-survey at Dhaka reported seropositivity of IgM Ab as 2% among 1128 healthy individuals (7). Yew et al. estimated the seroprevalence of dengue in blood samples collected during National Health Survey (NHS) and they found 2.6% positivity for IgM/high-titre IgG Abs (8). Ahmed et al. collected blood samples from 910 healthy adult male donors between March 2015

### Table 1. Period prevalence of dengue infection over 3 years

<table>
<thead>
<tr>
<th>NS1 only</th>
<th>IgM only</th>
<th>IgG only</th>
<th>NS1+IgG</th>
<th>IgM+IgG</th>
<th>Period prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>34</td>
<td>914</td>
<td>19</td>
<td>50</td>
<td>1032 (67.41%)</td>
</tr>
</tbody>
</table>

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and August 2016 and found 5.5% seropositivity for IgM Ab (1). Ranjan et al. conducted a study at the Institute of Liver and Biliary Sciences (ILBS), New Delhi for estimation of IgM Ab seropositivity among blood donors; they found 13.5% IgM Ab positivity (9).

Dengue IgG antibodies usually appear at day 7, peak at 2–3 weeks and persist for life. We found 64.21% (983) positivity for IgG Ab. There have been studies on the estimation of seroprevalence of dengue IgG Ab. Heffelfinger SB et al., on the basis of their sero-survey at Dhaka, reported seropositivity of IgG Ab as 80% among 1128 healthy individuals (7).

Zafar et al. estimated the seroprevalence of IgG Ab among healthy residents of Rawalpindi. Of 96 samples, they found 13.5% positive for IgG Ab (10). Mahmood et al. determined the seroprevalence of anti-dengue IgG antibodies in healthy adult population of Lahore. They found 67.2% IgG seropositivity (11). Mohsin et al. estimated the seroprevalence of past dengue infection in asymptomatic children of Lahore. Of 400 blood samples, 25% were found positive for IgG Ab (12). Jamjoom GA et al. estimated the seroprevalence of past dengue virus infection among healthy individuals of Saudi Arabia. Of 1939 subjects attending primary health care Centres, 47.8% were IgG-positive and of 184 blood donors, 36.95% were IgG-positive (13). Malavige et al. estimated the seroprevalence of dengue IgG among children up to 17 years of age with no evidence of a recent dengue infection or a family recollection of a dengue infection. Of 313 samples collected, 34.1% were positive for dengue IgG Ab (14). Yew et al. estimated the seroprevalence of dengue among adults in blood samples collected during NHS-2004 between September and December in Singapore. Of total 4152 samples, 59% were positive for dengue IgG infection (8). Chow et al. determined the seroprevalence of dengue virus infection in a cohort of healthy Singapore University undergraduates aged 19–26 years. Of 184 blood samples, 22% tested seropositive for dengue IgG Ab (15). L’Azou M et al. recruited 783 blood donors in the French West Indies tested their samples to determine the seroprevalence of dengue IgG. They found 93.5% positivity for IgG Ab (16). Yamashiro T et al. estimated the seroprevalence of dengue virus-specific IgG among 1008 blood donors and 201 children. Most of the adults (98%) and 56% of the children were positive for dengue virus-specific IgG (17).

**Conclusion**

Acute viraemia was detected by NS1 Ag seropositivity, which was 2.22% (34) with a peak point prevalence of 7.14% in October 2018. Antibody IgM Ab seropositivity was detected in about 5.49% (84) of subjects with a peak point prevalence of 14.29% in October 2018. IgG seropositivity was detected in 64.21% (983) throughout the year. Therefore, it is suggested that blood sample in blood bank should be tested for dengue (NS1Ag) before transfusion to prevent transfusion transmitted dengue infection as we estimated prevalence of active dengue infection varies from 2.22% to 7.17% by seropositivity of NS1 Ag among the samples of blood donors.
References


Are aptamers really promising for diagnostic or therapeutic applications in dengue?

Kapil Vashisht, Cherish Prashar, Shivangi Tyagi, Gunjan Rawat, Pratiksha Kumari, Kailash C. Pandey

ICMR-National Institute of Malaria Research, Delhi, India

Abstract

Dengue virus (DENV) has emerged as the fastest growing infectious virus, posing a considerable threat to ~40% of the world’s population. Ever increasing number of cases, and the first-time dengue reporting countries, is a result of viral transmission, being constantly fueled by global travel and climate change. The lack of anti-dengue therapy or vaccine for such an important disease warrants intensive efforts towards the development of diagnostic and therapeutic tools. Aptamers are promising biological molecules with striking advantages over traditional antibodies such as ease of selection, low cost of synthesis, high stability–specificity–sensitivity, potential for modifications and tagging, etc. Some important RNA/ssDNA/peptide aptamers have been highlighted in the present review, specifically focused on the viral diseases, viz. HIV, Zika and Japanese encephalitis virus (JEV). In the context of dengue, we have also collated a few promising aptamers which have been discovered in the current decade. This review also refers to our efforts in developing aptamers against non-structural proteins of dengue. As for any given new technology, it is worthwhile to explore the shortcomings, we have discussed the likely reasons for the lack of momentum in aptamer research against the dengue virus.

Keywords: Aptamers; antiviral; therapeutics; diagnostics; proteins

Introduction

In 2019, dengue was in the list of major diseases and recent outbreaks across the world confirmed this assumption. Dengue fever (DF) cases are currently being reported from 129 countries from the WHO regions of Africa, the Americas, South-East Asia and Western Pacific (1). The modelling data have predicted 390 million annual dengue infections, globally (2). The actual number of reported dengue cases at WHO rose from 505 430 (2000) to 3 312 040 (2015), a ~6-fold increase; whereas a majority of asymptomatic cases and varied national practices to record and report dengue cases would lead to underestimation of the global dengue burden. The gravity of the situation can be estimated from the record

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highest number of cases in 2019, globally with countries such as Afghanistan reporting dengue cases for the first time (3).

The transmission dynamics of dengue virus is crucial in occurrence of such high number of cases, which is ultimately dependent on its primary vector, the female Aedes mosquito. The vector – Aedes aegypti (day-time feeder) is compatible in urban habitats and breeds predominantly in man-made containers. Multiple feeding attempts before egg laying of the Aedes mosquito also add to the transmission capacity of dengue virus, circulating in hot-spot areas. Additionally, the presence of vector in geographical regions, previously naive to the vector as a result of climate change is also a great concern. DF is characterized by flu-like illness with symptoms such as severe headache, pain behind the eyes, muscle and joint pains, nausea, vomiting, swollen glands and rashes, etc. Escalation of symptoms may result in fatal complications from leaking of plasma, fluid accumulation, respiratory distress, severe bleeding and organ failure. Current practices of dengue detection involve virological methods (virus culture and reverse transcriptase-PCR) and serological methods (ELISA for detection of IgM and IgG anti-dengue antibodies). No specific treatment is available for dengue; however the first dengue vaccine (Dengvaxia® – CYD-TDV) has been under controlled trials with limited success (4). Thus, in view of the global disease burden and the absence of any specific treatment, it is imperative to develop highly sensitive and specific point-care diagnostic tests for dengue.

Pertaining to the context of current review, it is essential to discuss the fundamental aspects of aptamers in brief. RNA or ssDNA molecules with a specific conformational affinity towards target molecules are called – Aptamers. Identification of aptamers is most commonly achieved through a process known as SELEX (Systemic Evolution of Ligands by Exponential enrichment (5). A simplified schematic diagram of the SELEX technique is given in Fig. 1. A constant 5’ and 3’ end region acting as a primer and a variable sequence in between imparts the required diversity ($10^{12}$–$10^{18}$) in an aptamer library. A unique three-dimensional conformation of these sequences in certain experimental conditions (pH, ionic strength, temperature, etc.) or presence of ligand is the key characteristic of aptamers providing them the required specificity and affinity (6). A series of binding and washing steps are performed with the aptamer library and the target molecule, until the enrichment of a subpopulation of highly specific aptamers. These aptamers are then quantitatively and qualitatively validated by standard PCR and sequencing techniques. Aptamers have the following inherent advantages over the traditional antibodies: (i) vast range of physiological conditions for selection of desired aptamers; (ii) smaller/toxic proteins can also be targeted by aptamers; (iii) chemical synthesis ensures the identity, easy modification and tagging of reporter molecules; (iv) being oligonucleotides, aptamers detection by conventional methods; (v) regenerative capability; (vi) long-term storage; and (vii) efficient cellular entry by virtue of their smaller size.
Use of aptamers in viral diseases

Literature reviews of aptamer-based diagnostics and their use in viral diseases would signify their potential. Table 1 presents a collation of important aptamers against various target molecules from different viruses (HIV, Zika and JEV). In viral diseases, the maximum number of RNA/DNA aptamers have been discovered against HIV, with well-characterized interactions.
between aptamers and the viral genome or proteins. The binding of RNA aptamers against gag polyprotein inhibited virus production via interaction with matrix and nucleocapsid domains (7). An ssDNA aptamer was found to inhibit HIV-reverse transcriptase via abrogation of binding to other primer-templates (8). A complex of RNA aptamer and siRNA has been reported to suppress HIV viral load and rescue of CD4+ T cell count in humanized mice (9). Specifically modified anti-gp120 aptamer (with bridge sequence) used for delivery of cocktail of DsiRNAs (dicer substrate siRNAs) resulted in knockdown of the target mRNAs and further viral replication (10). Recently, Zika NS-1 binding ssDNA aptamers have been identified, which on coupling with Zika NS-1 antibody lowered the detection limit to 0.1 ng/ml in buffer (11). A peptide aptamer has also been identified from Zika virus envelope protein, which has further been shown to differentially detect Zika virus from dengue virus in serum and urine samples (12). RNA aptamers have also been found to inhibit the methyltransferase enzyme (JEV MTase) for one of the significant arbovirus disease – Japanese encephalitis virus (JEV) (13).

**Table 1.** Various aptamers against different target molecules in respective viral diseases

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Virus</th>
<th>Target molecule</th>
<th>Aptamer</th>
<th>Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HIV</td>
<td>gag polyprotein</td>
<td>RNA aptamer</td>
<td>Inhibition of viral replication (7)</td>
</tr>
<tr>
<td>2</td>
<td>HIV</td>
<td>Reverse transcriptase</td>
<td>ssDNA aptamer</td>
<td>Abrogation of binding other primer-templates (8)</td>
</tr>
<tr>
<td>3</td>
<td>HIV</td>
<td>gp120</td>
<td>RNA aptamer</td>
<td>Sequence-specific degradation of HIV RNAs (9)</td>
</tr>
<tr>
<td>4</td>
<td>HIV</td>
<td>gp120</td>
<td>DNA aptamer</td>
<td>Delivery of siRNA and knocking down of target mRNAs (10)</td>
</tr>
<tr>
<td>5</td>
<td>ZIKV</td>
<td>NS-1</td>
<td>ssDNA aptamer</td>
<td>Antibody coupling and heightened detection (11)</td>
</tr>
<tr>
<td>6</td>
<td>ZIKV</td>
<td>Envelope protein</td>
<td>Peptide aptamer</td>
<td>Differential detection of Zika from dengue in serum and urine samples (12)</td>
</tr>
<tr>
<td>7</td>
<td>JEV</td>
<td>Methyltransferase</td>
<td>RNA aptamer</td>
<td>Inhibited viral cap methylation activity (13)</td>
</tr>
</tbody>
</table>

**Aptamers in dengue research**

The characteristic properties (low cost of synthesis, high stability, specificity and sensitivity, etc.) of the aptamers make them valuable tools for research in diagnostic and therapeutic use for dengue as well. Table 2 presents a compilation of aptamers-based diagnostic and therapeutic applications in dengue. Notably, a modular biosensor consisting of oligonucleotide linker molecule after binding to complementary sequences of dengue genome, an aptamer/
Aptamers in dengue diagnostic and therapeutic applications

Restriction endonuclease signal transducer cleaves signaling molecules, generating fluorescence to confirm the presence of dengue sequences (14). In another development, aptamer (S15) can specifically bind to domain ED-III of the DENV-2 envelope protein. Further, S15 aptamer has been shown to be capable of neutralizing all the serotypes of DENVs, raising the hopes to develop a therapeutic aptamer for dengue (15). Recently, the DENV C protein has been shown to interact and co-localize with the host nucleolin (NCL) protein and this interaction can be disrupted by NCL binding aptamer (AS1411), resulting in reduction of viral titers (16). Interestingly, modified aptamers such as thio-aptamers have also been identified against the ED-III domain of the DENV envelope protein and further advocated to bind the ED-III in a similar fashion as antibody 1A1D-2, for virus neutralization (17). In a preliminary analysis, RNA aptamers binding to the 5’ UTR of the dengue genome have been identified, with potential use in antiviral agents and diagnostics (18).

Table 2. Various aptamers developed for diagnostic and therapeutic applications in dengue

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Target molecule</th>
<th>Aptamer</th>
<th>Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>DENV genome</td>
<td>DNA aptamer</td>
<td>Modular biosensor uses fluorescence quenching to detect viral genomic sequences (14)</td>
</tr>
<tr>
<td>2.</td>
<td>Interaction of DENV C protein and NCL of host protein</td>
<td>DNA aptamer</td>
<td>Disruption of interaction of NCL and DENV C protein significantly reduced viral titres (16)</td>
</tr>
<tr>
<td>3.</td>
<td>DENV E protein (ED-III)</td>
<td>DNA aptamer</td>
<td>Thioaptamer binding to ED-III for virus neutralization as 1A1D-2 antibody (17)</td>
</tr>
<tr>
<td>4.</td>
<td>DENV E protein (ED-III)</td>
<td>DNA aptamer</td>
<td>Binding to the domain ED-III and capable of neutralizing all serotypes of DENV (15)</td>
</tr>
<tr>
<td>5.</td>
<td>DENV genome</td>
<td>RNA aptamer</td>
<td>Interaction with functional RNA elements of the dengue genome (18)</td>
</tr>
</tbody>
</table>

Being in line with the current developments in aptamer research against dengue, our research group is also involved in the identification of high affinity aptamers against the crucial non-structural proteins of DENV. One of the target proteins is DENV NS-1, which is a 48 kDa glycoprotein and highly conserved among all flaviviruses. Due to the continuous secretion of NS-1 protein from the infected cells, it has been widely explored for diagnostic applications (19). Currently used diagnostic ELISA kits for acute stages of infection are based on DENV NS-1, with a variety of assays (20), and are faring better than the rapid diagnostic kits (RDTs). Thus, developing an aptamer-based diagnostic test to detect DENV NS-1 is worth investigating.
The promise of aptamers?

Despite the promising implications and exciting developments in the aptamer research, there are a few successful aptamer-based products available in the market. Macugen® is the only FDA (Food and Drug Administration)-approved aptamer against the vascular endothelial growth factor (VEGF)-165 for the treatment of macular degeneration (21). However, the promise of aptamers in disease diagnosis and therapeutics has some limitations, such as (i) degradation by host nucleases for oligonucleotide aptamers; (ii) small molecular renal clearance resulting in lower retention time for therapeutic use; (iii) aptamer interactions with intracellular off-targets due to cross-reactivity (22). Commercialization is paramount for sustained financial inputs in the research and development (R&D) of any given technology, leading to turnaround improvements from scientific community and the industry. The failure to expand the laboratory results of the aptamers to industrial scale and the difficulty in getting regulatory approvals are other reasons for not getting the right impetus in favour of aptamers. Additionally, huge financial commitments from the industry in the development of humanized monoclonal antibodies could be attributed to the lack of acceptance of aptamers in diagnostic and therapeutic use (23). However, more and more researchers are delving into the exciting world of aptamers, which has resulted in novel developments such as conjugation with nanoparticles, modified aptamers, etc. The renewed interest to develop highly functional and robust aptamers would be critical for the aptamers in competing with antibodies. As for dengue, the gap for effective antiviral therapy still exists and innovative techniques for improvement in specificity and sensitivity of aptamers would be promising.

Authors’ statement

The present review article has not been published and if accepted for publication in the Bulletin, will not be submitted for publication elsewhere without the agreement of WHO, and that the right of republication in any form is reserved by the WHO Regional Offices for South-East Asia and the Western Pacific.

Conflict of interest

The authors declare that there is no conflict of interest of any kind related to this manuscript.

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Aptamers in dengue diagnostic and therapeutic applications

References


Aptamers in dengue diagnostic and therapeutic applications


An integrated approach for control of Aedes breeding in the dump yard of articles confiscated by the Enforcement Department of South Zone of South Delhi Municipal Corporation: a case study

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Abstract

Introduction: Dengue has emerged as a major public health challenge in Delhi with high morbidity in the state. The present case study is an integration of multipronged approach to control and eliminate breeding sites of Aedes in articles confiscated by the South Zone of the South Delhi Municipal Corporation (SDMC) in collaboration with the National Institute of Malaria Research (NIMR), New Delhi for curtailing potential outbreaks.

Material and methods: This cross-sectional study was carried out at the dump yard of articles confiscated by the Enforcement Department of the South Zone, SDMC located at Kalu Sarai, in the vicinity of Azad Apartments, Kalu Sarai Village and near the campus of the Indian Institute of Technology (IIT), New Delhi. The store had more than 20,000 confiscated articles, which were seen holding water during monsoons in 2018. Mosquito larvae were collected from these refuge articles and sent to NIMR, where they were reared to emergence separately for species identification. Emerged (F1 generation) adult mosquitoes were pooled (each pool was up to ≤10 mosquitoes) according to sites were subjected to RNA extraction followed by RT-PCR for detection of dengue virus. Adult density was also calculated in surrounding households.

Results: A total of 1200 water-holding containers were checked and 720 water-holding containers were found positive for Aedes breeding (container index 60%). One pool of F1 generation of collected larvae of Aedes aegypti was found positive for DEN virus.

Conclusion: This study confirms that outbreak of dengue and chikungunya can be contained through a multipronged approach including anti-adult measures, antilarval measures, IEC (information, education, and communication) activities, source reduction, etc. and by using molecular techniques such as RT-PCR for early identification and action.

Keywords: Aedes; multipronged approach; confiscated articles; Municipal Corporation of Delhi; RT-PCR

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Introduction

Dengue fever is emerging as the most common vector-borne disease (VBD) in India. At present, all 28 states and 8 Union Territories of India are reporting dengue incidence (1, 2). Dengue has emerged as a major public health challenge in Delhi with outbreaks of dengue cases reported in a cyclic pattern. Unplanned urbanization and increase in population have increased the risk of dengue and other VBDs in Delhi. This is due to constraints on civic amenities, intermittent supply of water which is stored by the community in all sort of containers without proper lids, lack of proper solid waste collection and management, excessive use of plastic disposables, unused waste tyres, tourism and travel and evolution of dengue virus (3). Other factors that contribute to the dengue burden are high population density, i.e., population density of Delhi has increased from 9340 persons per sq.km during 2001 to 11 320 persons per sq.km in 2011 (4) mixed population and their movement along with changing epidemiology. This is attributed to water storage practices in different containers and such agglomeration have multiple containers. (5).

As per the guidelines of the National Vector Borne Disease Control Programme (NVBDCP), Government of India, civic bodies are responsible for taking measures for prevention and control of VBDs (1) in urban areas. In Delhi, following the trifurcation of erstwhile Municipal Corporation of Delhi, the NVBDCP is implemented by the South Delhi Municipal Corporation (SDMC), North Delhi Municipal Corporation and East Delhi Municipal Corporation in the areas under their respective jurisdiction. The total area of Delhi is approximately 1484 sq.km and the SDMC covers an area of 656.91 sq.km. Administratively, the SDMC is divided into four zones, i.e., Central Zone, South Zone, West Zone and Najafgarh Zone with 104 wards; and it provides civic amenities to about 70 lakh citizens (6). The SDMC provides civic amenities in diverse areas that include organized and planned residential areas, commercial establishments, rural and urbanized village areas along with resettlement and unauthorized colonies.

In Delhi, outbreaks of dengue fever have been reported in 2006, 2010, 2013 and 2015, which shows a break in the cyclic pattern and that the gap between outbreak years is decreasing (7). In 1996, a total of 10 252 dengue cases were reported from Delhi and the case fatality rate (CFR) was 4.2% (8–10), which declined in 2003, when 3366 cases and 38 deaths due to dengue were reported (11). In 2015, the cases and deaths were 15 867 and 60, respectively, and the CFR was 0.38%. The decline in the CFR can be attributed to better health-care facilities for management of severe cases of dengue and early health-seeking behaviour (Fig. 1).

The Public Health Department, SDMC is continuously taking measures for prevention and control of VBDs as per the guidelines of NVBDCP. Some major steps taken are: disease surveillance, entomological surveillance, vector control measures (regular anti-larval measures, anti-adult measures), environmental management, i.e. source reduction, capacity building, community behavior change, stakeholders’ involvement, law enforcement and operational
research. In addition, a zonal rapid response team was constituted to carry out epidemiological and entomological surveillance. *Ae. aegypti* is a major vector for transmission of dengue and chikungunya in Delhi. *Ae. aegypti* is a container-breeder and breeds in all domestic and peridomestic areas of different socio-economic groups in Delhi (12). Apart from domestic and peridomestic areas, solid waste dumps, stores of engineering department, workshops, bus depots and *malkahana* (store for seized items, vehicles, etc.) of the police department are other common sites for breeding of *Aedes* mosquito. For effective prevention and control of breeding, it was required to elicit support of different stakeholders.

**Fig. 1.** Year-wise dengue cases and case fatality rates (CFRs) reported in Delhi, 2006–Nov 2018

![Graph showing year-wise dengue cases and CFRs](image)

**Materials and methods**

**Study design:** This cross-sectional study was carried out from July 2018 to November 2018.

**Study area:** The study area was Kalu Sarai under Hauz Khas ward of the South Zone, SDMC. An open dump yard is located where articles confiscated by the Enforcement Department are stored. The area was chosen as frequent complaints of mosquito nuisance were received from the residents. Six sites (site 1–6) were selected randomly around the dump yard.

**Entomological surveillance and virus detection:** On receipt of complaints, entomological surveillance was carried out in the area. Multiple confiscated containers, solid waste articles, etc. were found in the store. These articles had collected rainwater in them. About 1200 containers/articles were searched, and 720 containers were found to be positive for mosquito breeding. Mosquito larvae were collected from these sites in plastic bags and transported to the National Institute of Malaria Research (NIMR) for rearing and emergence into adults,
and then for screening them for dengue virus and identification of species. Detection of virus in F1 generations was tested for DENV for any possibility of transovarial transmission. The size of each pool was ≤10 mosquitoes only. However, if collected mosquitoes in a house exceeded more than 10, the pools were increased. RNA extraction was done in pools using Qiagen kit (QIAamp Viral RNA mini kit) (13).

Reverse transcription polymerase chain reaction (RT-PCR) was done for detecting DENV using the standard protocol after Lanciotti et al. (1992). (13) The viral RNA was converted to cDNA and copies of a portion of the viral genome (capsid/prM) were produced and amplified using reverse transcriptase in the first step at (50 °C for 30 min) and in the second step two primers – forward primers (D1): 5’-TCAATATGCTGAACGCAGGAGAAACCG-3’, reverse primer (D2): 5’-TTGCACCAACAGTCAATGCTTTCCAGGTTC-3’– were used. The amplified product was visualized directly in 2% agarose gel electrophoresis (14). No serotyping was done to identify DENV type.

Adult density was carried out and man per hour density (MHD) was calculated in surrounding households of the store. Adult mosquitoes were collected using aspiratory tube.

**Variables in analysis and the analysis:** The variables in the present study were containers observed, positive containers among the total number of containers, number of pools of mosquitoes (male and female), virus isolated. Data were analyzed using percentages and proportions.

The present case study is unique where an integrated multipronged implementation approach (integrated vector management) was carried out for mosquito breeding control and source reduction. This study collaborated with NIMR (a premier institute) for the molecular technique, i.e., RT-PCR assay for detection of dengue and chikungunya virus (both RNA virus) as a surveillance tool. This multipronged approach helped to curtail the transmission of dengue/chikungunya in the area.

**Results**

The Enforcement Department uses this dump yard store to dump all confiscated articles in the open. In 2018, Delhi experienced rains in last week of July and rainwater was collected in these articles lying in the open. All the articles dumped in the store were not accessible due to overcrowding. A total of 1200 articles, which had collected water, were checked and 720 articles were found positive for mosquito breeding (container index 60%). All larval stages (1–4) and pupae were found during entomological surveillance. More than 80 adults female Aedes mosquitoes were collected from surrounding households and were found to be 80% MHD. Finding of such high larval breeding index and MHD was taken seriously, and the challenge was how to control this breeding as it may lead to massive outbreak in the area.
On inspection of the prepared pools F1 generation adult specimen at the NIMR, virus through RT-PCR (Table 1 & Fig 1).

**Table 1.** Results of screening of F-1, female Ae. Aegypti for dengue virus collected from Kalu Sarai, South Zone, SDMC

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Area</th>
<th>No. of F-1 females (Aedes aegypti)</th>
<th>No. of PCR pools tested by RT-PCR</th>
<th>No. of pools positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Site1</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>Site2</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>Site3</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4.</td>
<td>Site4</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5.</td>
<td>Site5</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>6.</td>
<td>Site6</td>
<td>02</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>52</td>
<td>06</td>
<td>1</td>
</tr>
</tbody>
</table>

**Fig 2.** Gel image showing dengue positive (lanes 2 and 4 with ladder of 100 bp at lanes 1 and 12; lane 11 is negative control)

**Table 2.** Status of entomological indices pre- and post-multipronged action

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Entomological index</th>
<th>Pre-multipronged action</th>
<th>Post-multipronged action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>House index (locality)</td>
<td>10%</td>
<td>1.5%</td>
</tr>
<tr>
<td>2</td>
<td>Container index (store)</td>
<td>60%</td>
<td>Nil</td>
</tr>
<tr>
<td>3</td>
<td>Manhourdensity</td>
<td>80%</td>
<td>3%</td>
</tr>
</tbody>
</table>
An integrated approach for control of Aedes breeding

Plate 1. Dump yard of confiscated articles, South Zone, SDMC
Plate 2. Entomological surveillance under supervision of an entomologist

Plate 3. Situation analysis by the Municipal Councilor and Deputy Health Officer
Plate 4. Anti-adults measures by focal spray and fogging

Plate 5. Antilarval measures
Plate 6. Post-multipronged action

Plate 7. Checking of domestic breeding in households
Plate 8. Map of Kalu Sarai
An integrated approach for control of Aedes breeding

Multipronged interventions by public health department  Entomological surveillance was carried out for larval and adults. We aimed to break down the transmission cycle through robust control using anti-larval operations, which was major a challenge due to the large size of the dump and countless breeding articles. Due to procedures involved in the government departments, it was not feasible to remove the articles. Therefore, the following multipronged measures were initiated (Table 2):

1. Household breeding and the surroundings were checked through domestic breeding checkers under supervision of the zonal entomologist.

2. Anti-adult measures were taken for immediate knockdown of adult Aedes mosquitoes to prevent fresh egg laying using focal fogging in the area using national programme-approved synthetic pyrethroid (Cyphenothrin) at the programme recommended dose (NVBDCP) (1). A hand-operated fogging machine was used for this operation. Indoor fogging was carried out in the surrounding area in a radius of 100 m. As a result, the total MHD dropped to 3%. The collected mosquitoes in this reduced MHD were mostly newly emerged.

3. Temephos 50% EC was sprayed at 1 ppm dose for anti larval operations using a vehicle-mounted pressurized pump sprayer (@ 20 cc per sq.m) with expert spray men of the corporation to avoid any wastage of insecticide.

4. A health education campaign was launched in the area by organizing meetings of the resident welfare associations, announcements were made through the public address system mounted on an autorickshaw, and handbills were distributed, and stickers were pasted.

5. To involve “stakeholders”, the staff posted in this store was directed to use personal protective measures. The Deputy Commissioner, South Zone, being the administrative head, was apprised of the situation and requested to issue directions to the officer in-charge of the Enforcement Department to immediately dispose of the articles through auctions. Each nodal person of the Enforcement Department was advised to ensure that collected water is removed before these articles were transported to a new dumping site. In three days, all articles were removed, and the store was cleared of the dumped articles.

6. A legal notice under malaria bye-laws 1975 of the DMC Act was issued by the malaria inspector in-charge of the concerned malaria circle to the staff posted at the store for creating mosquitogenic conditions with directions for source reduction by disposal of these articles. This legal notice had supplemented the directions issued by the deputy health officer to the enforcement Department for source reduction.

7. The municipal councilor (elected representative) of the local area was also involved in the entire operation to seek community support in taking care of mosquito breeding sites in and around their premises and observe personal prophylactic measures. Involvement of the municipal councilor generated political will and the community also extended their positive support though initially there was resistance.
Discussion

Stores, engineering workshops and police malkhanas always pose a challenge for breeding control in Delhi. This store was implicated as a major breeding ground after complaints of mosquito nuisance were received from local residents. Recent studies in Delhi have suggested that timely intervention, management of solid waste, water storing habits and awareness are key factors for control (15–18). Our integrated approach was the first of its kind that followed vector control in the dump yard/store of the Enforcement Department where all articles confiscated during raids are dumped in the open. After a multipronged intervention, there was no morbidity due to dengue/chikungunya in surrounding areas. No specific medicine and vaccines are available for dengue fever; hence entomological surveillance and source reduction are the key strategies for prevention and control of mosquito breeding, which has been shown in this study to significantly halt the outbreak of dengue.

It was ascertained by WHO that preventing or reducing the transmission of dengue virus depends entirely on the control of mosquito vectors or interrupting the human–vector contact. WHO ascertained that integrated vector management is the best strategy to control vectors in all VBDs, including dengue. The population of dengue vectors was controlled to a large extent by reducing their sources such as small containers in the premises of human dwellings. It is known that dengue control is possible only through advocacy, social mobilization and legislation, collaboration within the health sector and with other sectors, integrated approach to disease control, evidence-based decision-making and capacity building.

Good results can be obtained by community participation by eliminating water-filled containers for source reduction, which favour oviposition sites of Aedes mosquitoes, and antilarval work. The habitats are eliminated by preventing access to these containers or by emptying and cleaning them every week. In unavoidable situations, insecticides, biological control agents, or a combination of both are used. Evidence strongly supports the results of the present study in which an outbreak of dengue was averted. This approach was also adopted in the district of Tirunelveli in Tamil Nadu (19).

While traditional control strategies bring only temporary sustainability, it is advisable to improve their efficacy by implementing novel biotechnological interventions such as sterile insect techniques, Para transgenesis and production of genetically modified vectors.

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References


An integrated approach for control of Aedes breeding


Climate and dengue in the Pune region: prospects for an early warning system

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Abstract

This study aims to understand the influence of climatic factors on dengue occurrences in the Pune region, a Western Ghats mountainous district of India. Time series analyses revealed the seasonality and identified the lagged effects of rainfall, minimum and mean temperatures as the most influencing factors for dengue. A seasonal autoregressive integrated moving average model: SARIMA (1,1,1) × (0,0,1) 12, incorporating the meteorological parameters as external regressors, best described the dengue occurrence time series, was used. The model provided fairly effective forecast (projections) for the year 2017. The methodology developed can be used as an early warning system – ahead-of-season – for projection of dengue, which can be used by policy-makers to undertake preventive measures.

Keywords: Dengue; Western Ghats; Pune; India; EIP; climate; SARIMA

Introduction

Dengue, caused by dengue viruses, is a disease that has become endemic in more than 100 countries in Asia, America, Africa and the Western Pacific covering mostly the tropical zone. It is transmitted to humans by Aedes mosquitoes. The disease can be contracted by one of the four serotypes of the virus: dengue 1, dengue 2, dengue 3 and dengue 4 (1). It has been established that dengue transmissions are driven by complex interactions between human–hosts, mosquito–vectors and viruses that are influenced by environmental and climatic factors (1). Over the past decade, cyclic epidemics are increasing in frequency and in-country geographical expansion is occurring in India, Bangladesh and Maldives – countries in the deciduous dry and wet climatic zone with multiple virus serotypes circulating (WHO, 2009). Though environmental effects on dengue propagation are debatable, WHO has recognized dengue to be a climate-sensitive disease (2).

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In India, dengue is a notifiable disease and all confirmed cases are expected to be reported to the Government of India through the National Vector Borne Disease Control Programme (NVBDCP, 2018) (3). Between 2010 and 2017, a total of 600 042 people were affected by dengue with 1566 deaths across India (NVBDCP, 2018). India, due to its unique geographical position, has mostly tropical weather but the landmass is divided into the following climatic zones as per the Koppen classification: (i) Tropical wet (along the coastal plains and windward slopes of Western Ghats mountain range of peninsular India); (ii) Tropical wet and dry (major parts of peninsular India); (iii) Semi-arid steppe (west central India parts of Gujarat, Rajasthan and Madhya Pradesh); (iv) Humid subtropical (covering the Gangetic plains, central India and northeastern states); and (v) Mountainous climate covering the Himalayan regions (4,5). Climate of India is heavily influenced by the South-West monsoon (SW monsoon). During the SW monsoon season, moisture-laden winds from the Indian Ocean bring heavy rainfall over the landmass of India. Studies have shown effects of climate change over India as seasonal mean temperature has gradually increased in 100 years with 1.1 °C during winter and 0.9 °C during the post-monsoon season (6). Also, the geographical diversity of landmass created diverse climatic regions with varied ecosystems and vegetation (7). This diversity reflects in terms of insect propagation and seasonal abundance of mosquitoes (8,9). In any geographical location, agro-climatic factors drive mosquito propagation as well as distribution, degree of epidemicity of mosquito-borne diseases (10,11). Lot of demographic, socioeconomic factors and human activities have been found to contribute towards increase in vector-borne diseases in India (11).

Though the transmission of dengue in relation to climatic factors had been studied in Brazil (12–14), Bangladesh (15), Pakistan and other tropical countries (16–19), reports from India are sparse (15,20). The present study aims to assess the effects of meteorological factors on incidences of dengue in rural areas of Pune district, located in the Western Ghats mountainous region of India, with a view to develop a system ahead-of-season for projections of dengue.

**Methods**

**Study area and climate**

Pune district, part of the Maharashtra state, is located along the eastern slope of the Western Ghats mountain range. The average altitude of Pune district is 559 m (mean sea level: MSL). The district is located between 17°54’ to 19°24’ North and 73°29’ to 75°10’ East (Fig. 1) and covers a geographical area of 15 642 sq.km of which 1720 sq.km is occupied by forest and the gross cultivable area of the district is 10 150 sq.km (Central Ground Water Board, India, 2018). As per the Koppen classification, the district has a tropical wet and dry climate with rainfall occurring during the SW monsoon season (June–July–August–September: JJAS) and dry for rest of the year (4,21–23). Summer day temperature may be as high as 41 °C (April–May) and winter minimum temperature usually falls to 8 °C (January). There are four seasons for the Indian subcontinent: SW monsoon (JJAS), post-monsoon (October–November, an
intermediate between end of monsoon and winter), winter (December–January–February: DJF) and pre-monsoon season (March–April–May/June: MAM), till the SW monsoon arrives. Owing to its altitude, climate in Pune is moderately cold and wet during SW monsoon (average temperature ~25.3 °C for July/August), which is in contrast to other areas in the same latitude where SW monsoon season is hot and humid.

Fig. 1. Location of the study area in India. The image on the left indicates the state of Maharashtra; the inset shows Pune district

The topography of the study area is hilly terrain with a few river valleys at places (supplementary figures). The main crops cultivated are jowar, wheat, sugarcane, cotton, rice and variety of green vegetables and flowers in lesser quantities (24). Villages have moderate green cover, though dense woods are sparse. The higher mountainous terrain (>600 m MSL) and slopes are usually covered by grasslands.

Data collection

The monthly incidences of dengue (confirmed cases) in the population of rural regions of Pune district from 2000–2016 were obtained from the records of the Public Health Department, Government of Maharashtra.

Daily meteorological data for Pune for the period 2000 to 2016 have been procured from the National Data Centre, India Meteorological Department, Government of India. Averages and derived parameters were calculated by the authors. Mean temperature for every month was calculated from the maximum (MXT) and minimum (MNT) temperatures.
 Monthly El Nino-Southern Oscillation (ENSO) index is the Nino3.4 as available from the Climate Prediction Centre of the US National Weather Service (URL: http://www.cpc.ncep.noaa.gov/data/indices/sstoi.indices).

**Dengue external incubation period**

Outbreaks of dengue occur under environmental conditions that favour mosquito abundance and effective viral transmission by *Aedes* mosquitoes. The extrinsic incubation period (EIP) for dengue is defined as the time period between the virus entry into mosquito body and virus being readily available in saliva for transmission. At any geographical location, dengue EIP is dependent on the mean day temperature (25,26) and is vital for viral transmission by the vector mosquitoes.

The EIP for dengue was calculated as:

\[
EIP = \tau_e = 97.177 \, e^{-0.0795 \, T}
\]

Eqn. (1)

where, \( T \) is the mean temperature.

**Time series analyses and SARIMA models**

Simple time series analyses of data comparing dengue occurrences with meteorological parameters were performed in R, using the cross-correlation function (ccf). To understand the time series structure of the dengue occurrences and meteorological parameters, we used seasonal autoregressive integrated moving average (SARIMA) models. The SARIMA model is best described as:

\[
\text{SARIMA} (p, d, q) \times (P, D, Q)_S,
\]

where, \( p \) is the non-seasonal autoregressive (AR) order, \( d \) is the non-seasonal differencing, \( q \) is the non-seasonal moving average (MA) order, \( P \) is the seasonal autoregressive (SAR) order, \( D \) is the seasonal differencing, \( Q \) is the seasonal moving average (SMA) order represent the same notations for seasonal aspect and \( S \) represents the period of seasonal patterns (27–30).

Univariate seasonal ARIMA models were used to get the best time series structure for dengue occurrences using the “auto.arima” protocols as implemented in the R package. The best model was selected based on the minimum Akaike information criterion (AIC). A residual analyses was conducted to check the validity of the model assumptions. This involved time series plot of the residuals, residual auto-correlations and the Ljung-Box test (31,32). The best model with the lowest AIC also accounted for the residuals above 0.05, indicating the significant and best fitting.
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Multivariate SARIMA was conducted by incorporating the meteorological parameters as external regressors into the best dengue SARIMA model, using the “sarima” protocol as implemented in the “astsa” package of R. Based on the SARIMA fitting of data from 2000 to 2016, forecasts of dengue occurrences for the year 2017 were made. Details of R protocols have been provided in the supplementary materials.

The efficacy of our model-based forecast (projections) was evaluated in terms of mean absolute percentage error (MAPE) defines as:

$$MAPE = \frac{1}{n} \sum_{t=1}^{n} \left| \frac{x_t - x_f}{x_t} \right|$$

where $x_t$ is the reported number of monthly cases and $x_f$ are the forecasted number of monthly cases and $n$ is the number of months (33).

Software used

Data analyses and graph plotting has been carried out using MS Excel and statistical calculations for time series performed in R. All maps are in accordance with the map display norms of the Republic of India.

Survey in rural areas

To understand the topography and village ecosystems authors made a survey of 20 villages (which reported dengue cases between 2012 and 2016) in Pune, Maharashtra, India. Locations were selected based on freely available terrain maps from “Bhuvan” – the Indian Geo-platform of Indian Space Research Organization (ISRO) (URL: http://bhuvan.nrsc.gov.in/map/bhuvan/bhuvan2d.php) and Google maps (URL: https://www.google.co.in/maps/). Authors visited the villages for documenting the topography and land-use. The list of villages is given in Supplementary Table 1. Each location was surveyed thrice – once in each of the following seasons: winter (January), pre-monsoon (May) and SW monsoon (July).

Results

This study aimed to assess the role of environmental factors on incidences of dengue in rural areas of Pune district, India from 2000 to 2016. This is the first attempt towards development of an early warning system for dengue from the Western Ghats mountainous region of India.
Temperature and EIP

From the monthly average mean temperature (MT) we calculated the extrinsic incubation period (EIP) for dengue using equation (2). Fig. 2A compares the monthly dengue occurrences with monthly values of EIP. The EIP being dependent on MT showed variation over seasons but followed similar trends every year. The range of EIP for the Pune region is indicated in Fig. 2B. While the EIP for dengue appeared very low in April/May and remained lower, values ranged from 10 to 12 days during the SW monsoon seasons (2000–2016), highest values were achieved during winters.

*Fig. 2.* (A) EIP for dengue (monthly averages) and dengue monthly incidences (per 2 000 000 individuals) in rural Pune from 2000 to 2016. (B) Dengue EIP (days) estimated from daily temperature data from 2000 to 2016 for Pune district
Seasonal distribution of occurrences

The temporal monthly cases of dengue in rural Pune between 2000 and 2016 are plotted with temporal monthly rainfall in Fig. 3. Overall during the period of the study, on average 52% of cases occurred in the SW monsoon season (JJAS), followed by 28% in the post-monsoon season (ON), 13% in the pre-monsoon season (MAM) and only 7% in the winter season (DJF). In 2003, there have been incidences of dengue in the pre-monsoon season (April/May) followed by a surge of cases during the SW monsoon (JJAS) and post-monsoon seasons (ON). No cases were reported during the winter seasons (DJF). Similar trends were observed in 2007 and 2008. However, since 2012 onwards we observed an increase in incidences during the monsoon and post-monsoon seasons. In 2013, highest number of cases were reported in the post-monsoon season (October–November). In 2014, incidences were recorded during the pre-monsoon season (March–June) as well as during SW monsoon. Cases continued in the subsequent winter season (DJF, 2015). In 2015 and 2016, maximum number of incidences occurred towards the end of SW monsoon and post-monsoon seasons.

**Fig. 3.** Monthly dengue incidences (dengue cases per 20 00 000 individuals) and rainfall (2000–2016), rural Pune

Time series analyses and seasonal ARIMA models

A cross-correlation analyses applied to time series of monthly dengue cases and monthly rainfall (RF) revealed the highest positive correlation at lag 2 months, indicating the delayed influence of rainfall (Fig. 4). Also, positive correlations were observed between monthly dengue occurrences with: (i) maximum temperature (MXT) lagged by 6 months; (ii) minimum temperature (MNT) lagged by 2 months; and (iii) monthly mean temperature (MT) lagged by 4 months.
To understand the time series structure for dengue occurrences, data were fitted to SARIMA models using the “auto.arima” protocol of R, with the best model selected based on the minimum values of the Akaike information criterion (AIC).

The best-fit model for dengue occurrences was found to be the SARIMA $(1,1,1) \times (0,0,1)_{12}$ with coefficients for non-seasonal autoregressive order, $\text{AR}(1)=0.38$, moving average order, $\text{MA}(1)=-0.961$, seasonal $\text{MA}(1)=0.20$ and seasonal $\text{MA}(2)=0.156$. The residual analyses revealed the adequacy of the model, with uncorrelated residuals up to lag 10 ($P=0.6975$ from the Ljung-Box test (33) indicating significant results [$P>0.05$]). Fig. 5 shows the decomposition of the dengue time series (R package). This revealed the seasonality for dengue, as similar patterns were repeated in 12-month cycles.

Multivariate analyses were carried out by incorporating meteorological parameters (as external regressors) into the dengue SARIMA $(1,1,1) \times (0,0,1)_{12}$, using the “sarima” function.
as implemented in R package (please refer to supplementary materials for details). Since the environment is an interplay of various meteorological parameters taken together, the multivariate model was constructed by incorporating the parameters: MXT (6 months lag), MNT (2 months lag), MT (4 months lag) and RF (2 months lag) into the dengue SARIMA. The outputs from this model are summarized in Table 1. The plots of the standardized residuals and Ljung-Box tests are included in the supplementary materials. The results were compared with a model without the regressors (NULL model). The NULL model recorded a higher AIC value compared to the one with the meteorological parameters. Since the multivariate SARIMA model was found appropriate (lowest AIC), it was then used for forecasting dengue incidences in rural Pune for the year 2017.

**Fig. 5.** Annual dengue incidences and annual rainfall for rural Pune (2000–2016)

<table>
<thead>
<tr>
<th>SARIMA models</th>
<th>Parameters</th>
<th>Estimate or coefficient</th>
<th>SE</th>
<th>P value</th>
<th>AIC</th>
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<td>(meteorological parameters as regressors)</td>
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<tr>
<td></td>
<td>sma1</td>
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<td></td>
<td>MNT_lag2</td>
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<tr>
<td></td>
<td>MT_lag4</td>
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<td></td>
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<td>NULL regressors</td>
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<td>sma1</td>
<td>0.1858</td>
<td>0.07</td>
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</tbody>
</table>

Table 1. Estimates, standard error (SE) and P values of the dengue SARIMA models
Finally, for validation, the forecast results (predD) were compared with the reported confirmed cases in 2017. The data were provided by the Public Health Department, Government of Maharashtra. A two-sample t-test conducted comparing the reported number of occurrences in 2017 (D) and forecasted number of occurrences (predD) for the June–November period (JJAS-ON) indicated that the two series are not significantly different (t=0, df=10, P-value=1; vide supplementary materials for details). A non-parametric Wilcoxon’s test (unpaired) also revealed that the two series are not significantly different (P=0.8099, P>0.05). Fig. 7A describes the SARIMA fitting of dengue occurrences and the projections for 2017, while the Fig. 7B compares the forecast values (projected cases, predD) with the reported number of cases for the SW monsoon season (JJAS) and post-monsoon season (ON). Mean absolute percentage error (MAPE) between the time series for forecast values (predD) and reported dengue incidences was estimated. A very low value of MAPE=1.078 indicated the efficacy of the forecasting model.

**Terrain, annual rainfall and water management**

Fig. 6 compares the annual dengue incidences with annual rainfall. Data indicate that in rural Pune the dengue incidences were least in 2005, 2006, 2010 and 2011 A.D, the years with high annual rainfall (>1000 mm). In the years of moderate to strong positive El Nino Southern Oscillation (ENSO) (2002, 2003, 2007, 2008, 2013–2016) with less annual rainfall (RF≤800 mm), there had been surge in dengue incidences. A moderate negative association between annual RF and dengue cases (Pearson’s coefficient, r=-0.44), can be attributed to topography and environment, land use and human activities. This result is consistent with reported relationship between annual rainfall and annual dengue incidences for the peninsular India (34).

A survey of 20 villages were conducted across Pune district to record the geographical and ecological features as well as water flow patterns. Based on the observations, we classified the rural human habitations in the study area broadly into two types: (i) **hillside** villages – those located along slopes of hills (approximately 1 km away from any river or stream); and (ii) **streamside** villages – those located in the valleys and/or in watershed areas of rivers, within 1 km from river /irrigational canal/major stream. Supplementary Table 1 gives the names of the villages surveyed. Photographic evidence of the terrain is provided in Supplementary Figs. 2–6. Most hillside villages practice monsoon farming on terraced fields and depend on portable water supply for the dry seasons. The streamside villages practice cultivation in larger scale, but still depend on portable water supply for household needs during the dry seasons. A majority of households (92%) have large or small tanks for harvesting or storage of water. Unpublished data from authors’ laboratory (mosquito survey in rural Pune) revealed the presence of five species of Aedes including *Aedes albopictus* and *Aedes aegypti*, both vectors of dengue. The survey also revealed the water management practices in these villages. Supplementary Fig. 7 shows schematic diagrams of hillside and streamside villages indicating the water flow pattern during monsoon rainfall. Thus, in heavy rainfall years (RF≥1000 mm) washout of the natural breeding habitats in hilly terrain (terraced fields) combined with lesser
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**Discussion**

India has a huge burden of dengue and chikungunya with states of peninsular India, viz. Maharashtra and Karnataka, reporting high numbers of cases (35) over the past 10 years. Considering that dengue has spread to rural settings in India (34,36), it is crucial to develop
a forecasting model that can provide projections for the next season. The present study aims to understand the influence of meteorological factors on the dengue incidences in the Western Ghats mountainous region of rural Pune and develop an early warning system that can generate ahead-of-season projections. Vector propagation and competence to VBD transmission depend on climatological factors as well as availability of habitat (37). Thus, both climatic factors as well as the landscape and ecology play an important role in vector propagation and dengue transmission. Our analyses on seasonal distribution of cases revealed that highest incidences (52%) occur during SW monsoon in rural Pune. In this season, the dengue EIP remains low, which indicates that the infected mosquitoes become ready to transmit within a short time of 8–10 days since the intake of dengue-infected blood (25,26).

Mosquito surveys conducted in the Pune region have established that climatic conditions remain favourable for high Aedes mosquito abundance during the SW monsoon season due to the low value of diurnal temperature range (DTR) – the difference between maximum and minimum temperatures in a day (8,38). Though the least value of EIP was observed in April–end/May each year, the cases during these months were few. This is because of the prevailing hot and dry weather conditions, which might have resulted in lower abundance of Aedes mosquitoes (8). During SW monsoon, which favours high mosquito abundance and longevity, the occurrence of lower EIP (due to moderate mean temperature) would enhance the chances of dengue transmission.

A survey conducted by the authors’ institution in villages in the Pune region in 2016–17 revealed the presence of Ae. albopictus as well as Ae. aegypti, both vectors for dengue. Ae. aegypti were found in a majority of the households surveyed. In heavy rainfall years, people store less water compared to the less rainfall years. Thus, lower rainfall years (RF≤800 mm) would enhance breeding of Ae. aegypti in water storage facilities (farmstead and domestic). Also, in the lesser rainfall years, the chances of washout of the outdoor breeding habitats for Ae. albopictus are relatively lesser. In the heavy rainfall years, water flows in cascades down the mountainous terrain causing washout of the breeding habitats for Ae. albopictus. Supplementary Fig. 7 explains the geological features for water-flow. Thus, it is clear that lesser rainfall was favouring both the Aedes species and consequently enhancing the dengue occurrences compared to the heavy rainfall (RF≥1000 mm).

Rainfall in the Pune region occurs mostly due to the SW monsoon winds. Monsoon rainfall over the Indian subcontinent is affected by ENSO, which corresponds to an irregularly periodic variation in sea surface temperatures (SST) and wind circulation over the tropical part of the eastern Pacific Ocean. The erratic increase in the SST is called El Nino while, Southern Oscillation (SO) corresponds to the associated wind circulations and SST changes in the surrounding regions (NOAA, USA URL: origin.cpc.ncep.noaa.gov/). The ENSO is a well-defined climate phenomenon that periodically fluctuates between three phases: Neutral, La Niña or El Niño. A positive ENSO (El Nino) adversely affects SW monsoon circulation and reduces rainfall over India causing droughts (39), whereas a neutral or negative ENSO (La Nina) favours Indian SW monsoon rainfall. That ENSO-driven rainfall anomaly affects
dengue transmission, which has been well established in various countries such as Ecuador, Bangladesh and China (2,15,40). However, some studies have found weak or no correlation between ENSO and dengue, because ENSO affects the local climate in varied ways (41,42). We found that in rural Pune the high rainfall years recorded lesser number of dengue cases compared to positive ENSO years with lesser rainfall. This is because in the positive ENSO years with rainfall ≤800 mm, minimal habitat washout (for Ae. albopictus) and water storage practices of humans enhanced breeding of Ae. aegypti. In contrast, in high rainfall years the storage is minimal, and the chances of habitat washout are also high (43). Our observations are similar to the findings elsewhere in India and other tropical Asian countries, viz. Indonesia, Myanmar and Thailand, where piped or portable water supply and scarcity of water led to enhanced water storage and resulted in exacerbation of dengue (World Health Organization). Similar modulation of dengue outbreaks and incidences by rainfall and water storage practices have been documented in Sri Lanka (44). This is consistent with findings elsewhere that rainfall influences active breeding of Aedes mosquitoes leading to greater mosquito abundance preceding a dengue outbreak (45,46).

Looking ahead to future disease prevalence scenarios helps in decision-making and taking preventive measures. Thus, tools of time series analyses have been widely used (Table 2). Advanced time series modelling with SARIMA has emerged as a powerful tool for analyses of environmental factors on disease occurrences (28). In this study we considered best-fit univariate SARIMA (1,1,1,) × (0,0,1)12 based on minimum AIC to describe the dengue time series. It is evident that occurrences are influenced by the number of occurrences in the previous month (AR=1), and seasonality was established with similar patterns (peaks) repeated every 12 months. The lagged effects of meteorological factors on monthly dengue incidences were incorporated into the dengue SARIMA as external regressors: monthly rainfall (2 months lag), maximum temperature (6 months lag), mean temperature (4 months lag) and minimum temperature (2 months lag). This result is biologically relevant considering that monthly rainfall and temperature ranges are known to influence mosquito abundance and disease propagation (47,48). While monthly rainfall affects Aedes mosquito propagation, the temperature factors help sustain the adult mosquito abundance (survival and longevity) by influencing mosquito biology (47,48). Prerequisites for the spread of dengue at any geographical location include: (i) abundance of Aedes mosquito population; and (ii) temperature factors favourable for low dengue EIP (effective transmission). Unpublished data on random mosquito surveys available at the National Institute of Virology, Pune revealed the presence of both Ae. aegypti and Ae. albopictus, potential vectors for dengue. Vector-borne disease transmission is a complex interplay of various factors including host–pathogen, vector–host interactions as well as influence of environmental factors on vector population. Moderate mean temperatures (~25 °C) favour mosquito survival and longevity in nature. The longer the mosquito survived, the greater is the probability that it would have more than one blood meal and consequently, there is a greater possibility of disease transmission as well as breeding. It has been observed that during the SW monsoon season a lower diurnal temperature range (DTR) – the difference between maximum and minimum temperatures – favours survival and abundance of mosquitoes in the Pune region (8). Thus, the mosquito
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population at any point of time depends on the survival of their ancestors in the immediate past. Hence, an association of disease with mean temperature (4 months lag) should not appear as a surprise as higher mosquito abundance precedes disease outbreaks (45).

Table 2. Various SARIMA models used to describe dengue in different countries

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Data type</th>
<th>Model</th>
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<tbody>
<tr>
<td>Hu et al. (53)</td>
<td>Queensland, Australia</td>
<td>Monthly (1993–2003)</td>
<td>SARIMA(1,0,0)(2,1,0)$_{12}$</td>
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<td>Gharbi et al. (54)</td>
<td>French West Indies</td>
<td>Weekly (2000–2006)</td>
<td>SARIMA(0,1,1)(0,1,1)$_{52}$</td>
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<tr>
<td>Martinez et al. (55)</td>
<td>Ribeirao, Preto, Brazil</td>
<td>Monthly (2000–2008)</td>
<td>SARIMA(2,1,3)(1,1,1)$_{12}$</td>
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<tr>
<td>Dela Cruz et al. (56)</td>
<td>The Philippines</td>
<td>Monthly (2005–2010)</td>
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<tr>
<td>Phung et al. (57)</td>
<td>Viet Nam</td>
<td>Monthly (2003–2010)</td>
<td>SARIMA(1,1,1)(1,1,0)$_{12}$</td>
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<tr>
<td>Luz et al. (58)</td>
<td>Rio de Janerio, Brazil</td>
<td>Monthly (1994–2004)</td>
<td>SARIMA(2,0,0)(1,0,0)$_{12}$</td>
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<td>Our results</td>
<td>Pune region, India</td>
<td>Monthly (2000–2016)</td>
<td>SARIMA(1,1,1)(0,0,1)$_{12}$</td>
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Time series analyses incorporating climate variables as external regressors into SARIMA models have been more accurate compared to those without (Table 2) (49,50). In the present study, the multivariate SARIMA model incorporating the meteorological factors was used to forecast dengue occurrences for 2017. Since any forecasting model assumes that a distribution pattern will repeat in future, the forecast for 2017 followed similar trends as for the previous years. Predictions have closely matched with the number of reported dengue occurrences in 2017 (from June to September). Thus, in general, our model could effectively forecast disease incidences. In October, the reported number of confirmed cases was found to be higher than that projected by our model (Fig. 7B). This is because September and October 2017 had experienced a deviation of weather conditions from the previous years’ trends. Unusual higher rainfall (141 mm) was recorded in September 2017 (27 rainy days) compared to average (2006–2016) value of 86 mm (21 rainy days). October 2017 also recorded higher rainfall (98 mm, 19 rainy days) compared to the average value of 77 mm (5 rainy days) [as per online Weather Atlas; URL: https://www.weather-ind.com/en/india/pune-climate]. The withdrawal of monsoon was delayed to 26 October 2017. The extended monsoon, coupled with moderate mean temperature (~25 °C), may have favoured *Aedes* abundance and dengue transmission, thus generating more cases in October 2017 compared to previous years.

The present study revealed that the annual number of confirmed cases (occurrences) in 2013–2016 have increased to almost twice that of 2007–2008. The rural population has grown from 3 039 856 in 2001 to 3 678 226 in 2011, i.e. 21% increase with least inward migration and some outward migration (51). Also, the inward migrations of people to Pune
rural areas from other states is negligible since migrant labourers, students and professionals migrated to Pune urban zone only over the past two decades (52). Hence, the increase in the number of occurrences in rural Pune cannot be attributed to human demographics. Also, it should be noted that for the years 2013–2016, the total annual rainfall was ≤800 mm, but the monsoon withdrawals were late (between 17 and 26 October compared to the “normal” date of 1 October) from the Pune region (Supplementary Table 2). The late withdrawal resulted in more number of rainy days and sustenance of humid monsoon-like weather conditions for extended periods of time, thus favouring propagation of dengue. In other words, light rain distributed over longer periods of time may have favoured sustained Aedes mosquito abundance and enhanced the dengue occurrences in these years.

With its unique climate, experiencing cold and damp SW monsoon, Pune district stands apart from other districts of India in the same latitude. Our study demonstrated the influence of climate factors on monthly dengue occurrences in rural Pune. SW monsoon season was found to be most favourable, with most number of cases every year. The SARIMA model gave a fair projection of dengue occurrences for the 2017 SW monsoon season. Our estimates of lagged effects are relevant in terms of the biology of the vector and virus. Overall, the study improves our understanding of the role of climate factors in driving the dengue occurrences in rural Pune. Methods developed in the study can be used as an early warning system for ahead-of-season projection of dengue, which can be used by policy-makers for undertaking control measures.

**Conclusion**

The present study explained the influence of climate factors on the occurrences of dengue in the Pune region located in the Western Ghats mountains of India. The mathematical model developed in the study provided a good ahead-of-season projection (prediction) for dengue occurrences. This could be used as an early warning system for dengue. The methodology developed can be used to study the influence of climate on dengue in different parts of India.

**References**


Climate and dengue in the Pune region: prospects for an early warning system


[55] Martinez EZ, Silva EA. Predicting the number of cases of dengue infection in Ribeirão Preto, São Paulo State, Brazil, using a SARIMA model. Cad Saude Publica. 2011; 27(9):1809-18.


Supplementary materials

**Supplementary Fig. 1.** Map of Pune District, courtesy Google Maps

**Supplementary Fig. 2.** Hilly villages in rural Pune, eastern slopes of the Western Ghats mountain range
Supplementary Fig. 3. Hilly villages in rural Pune (monsoon 2018)

Supplementary Fig. 4 A and B. Hilly villages in rural Pune, terraced fields for cultivation (monsoon 2018)
Supplementary Fig. 5. Streamside villages in rural Pune

Supplementary Fig. 6 A, B, C. Typical landscapes of the Western Ghats. Images taken during SW monsoon season 2018 (July–September)
Climate and dengue in the Pune region: prospects for an early warning system
**Supplementary Fig. 7.** Artistic impressions of village landscapes in Pune district: (A) hillside and (B) streamside

**Supplementary Table 1.** List of villages surveyed

<table>
<thead>
<tr>
<th>Name of village</th>
<th>Type</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sus (Pune)</td>
<td>Hillside</td>
<td>Partially terraced</td>
</tr>
<tr>
<td>Kandi</td>
<td>Hillside</td>
<td>Terraced</td>
</tr>
<tr>
<td>Darawadi</td>
<td>Hillside</td>
<td>Terraced</td>
</tr>
<tr>
<td>Shelarwadi (Dehu Road)</td>
<td>Hillside</td>
<td>Undulating, partially terraced</td>
</tr>
<tr>
<td>AdheKhad (Maval)</td>
<td>Streamside</td>
<td>Undulating landscape, small streams</td>
</tr>
<tr>
<td>Urse</td>
<td>Streamside</td>
<td>Pawana River watershed</td>
</tr>
<tr>
<td>Pirangut</td>
<td>Streamside</td>
<td>Mula River watershed</td>
</tr>
<tr>
<td>Koregaon-Bhima</td>
<td>Streamside</td>
<td>Bhima River watershed</td>
</tr>
<tr>
<td>Sonwadi (Daund)</td>
<td>Streamside</td>
<td>Bhima River watershed</td>
</tr>
<tr>
<td>Talegaon</td>
<td>Hillside</td>
<td>Populated localities, floriculture</td>
</tr>
<tr>
<td>Rui (Baramati)</td>
<td>Streamside</td>
<td>Karha River watershed</td>
</tr>
<tr>
<td>Name of village</td>
<td>Type</td>
<td>Remarks</td>
</tr>
<tr>
<td>-----------------</td>
<td>------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>12 New Khandi</td>
<td>Hillside</td>
<td>Terraced and woodlands</td>
</tr>
<tr>
<td>13 Karla</td>
<td>Streamside</td>
<td>Indryani River watershed</td>
</tr>
<tr>
<td>14 Chandkhed</td>
<td>Streamside</td>
<td>Undulating landscape</td>
</tr>
<tr>
<td>15 Paud</td>
<td>Streamside</td>
<td>Mula River watershed</td>
</tr>
<tr>
<td>16 Mahabaleshwar</td>
<td>Hillside</td>
<td>Forested, terraced, fruit cultivation</td>
</tr>
<tr>
<td>17 Chikhali</td>
<td>Streamside</td>
<td>Mutha River watershed</td>
</tr>
<tr>
<td>18 Belawade</td>
<td>Hillside</td>
<td>Terraced</td>
</tr>
<tr>
<td>19 Maded</td>
<td>Hillside</td>
<td>Terraced</td>
</tr>
<tr>
<td>20 Baramati</td>
<td>Streamside</td>
<td>Karha River watershed</td>
</tr>
</tbody>
</table>

**Supplementary Table 2.** Actual dates of SW monsoon withdrawal from the Pune region

<table>
<thead>
<tr>
<th>Year</th>
<th>Date of SW monsoon withdrawal from Pune region</th>
<th>Source of information</th>
</tr>
</thead>
</table>
Mathematical analyses and statistical modelling

SEASONAL ARIMA ANALYSES: OUTPUTS FROM R

> auto.arima(mydata$Den, trace=TRUE, test="kpss", ic= "aic")

Fitting models using approximations to speed things up...

ARIMA(2,1,2)(1,0,1)[12] with drift : Inf
ARIMA(0,1,0) with drift : 1475.983
ARIMA(1,1,0)(1,0,0)[12] with drift : 1470.862
ARIMA(0,1,1)(0,0,1)[12] with drift : Inf
ARIMA(0,1,0) : 1473.984
ARIMA(1,1,0) with drift : 1463.18
ARIMA(1,1,0)(0,0,1)[12] with drift : 1460.383
ARIMA(1,1,0)(1,0,2)[12] with drift : 1473.006
ARIMA(0,1,0)(0,0,1)[12] with drift : 1476.501
ARIMA(2,1,1)(0,0,1)[12] with drift : 1458.794
ARIMA(2,1,1)(0,0,1) with drift : Inf
ARIMA(3,1,1)(0,0,1)[12] with drift : 1425.804
ARIMA(3,1,1)(0,0,1)[12] : 1425.602
ARIMA(3,1,1)(1,0,1)[12] : 1437.338
ARIMA(3,1,1) : Inf
ARIMA(3,1,1)(0,0,2)[12] : 1424.106
ARIMA(2,1,1)(0,0,2)[12] : 1422.197
ARIMA(2,1,0)(0,0,2)[12] : 1455.047
ARIMA(2,1,2)(0,0,2)[12] : 1423.98
ARIMA(1,1,0)(0,0,2)[12] : 1457.494
ARIMA(3,1,2)(0,0,2)[12] : 1425.293
ARIMA(2,1,1)(0,0,2)[12] with drift : Inf
ARIMA(2,1,1)(1,0,2)[12] : 1436.026
ARIMA(2,1,1)(0,0,1)[12] : 1424.133
ARIMA(1,1,1)(0,0,2)[12] : 1419.261
ARIMA(1,1,2)(0,0,2)[12] : 1421.211
ARIMA(0,1,0)(0,0,2)[12] : 1472.772
ARIMA(1,1,1)(0,0,2)[12] with drift : Inf
ARIMA(1,1,1)(1,0,2)[12] : 1433.083
ARIMA(1,1,1)(0,0,1)[12] : 1421.333
ARIMA(0,1,1)(0,0,2)[12] : 1439.284
Now re-fitting the best model(s) without approximations...

\text{ARIMA}(1,1,1)(0,0,2)[12] \quad : 1425.982

Best model: \text{ARIMA}(1,1,1)(0,0,2)[12]

Series: mydata$Den

\text{ARIMA}(1,1,1)(0,0,2)[12]

Coefficients:

\begin{verbatim}
ar1  ma1  sma1  sma2
0.3874  -0.9677  0.2053  0.1560
\end{verbatim}

s.e.  0.0764   0.0203  0.0814  0.0816

\text{sigma}^2 \text{ estimated as } 160.9: \text{ log likelihood} = -707.99

\text{AIC}=1425.98   \text{ AICc}=1426.33   \text{ BIC}=1441.92

Multivariate sarima incorporating the climate factors as xreg.

\text{SARIMA} (1,1,1) (0,0,1) 12 for \text{Den} with xreg \rightarrow \text{climate parameters (variables that affect Den)}

\text{>sarima}(\text{mydata}$Den, 1, 1, 1, 0, 0, 1, 12, \text{xreg= sdata})

Call:

\text{stats::arima(x = xdata, order = c(p, d, q), seasonal = list(order = c(P, D,}
\text{ Q), period = S), xreg = xreg, optim.control = list(trace = trc, REPORT = 1,}
\text{ reltol = tol))}

Coefficients:

\begin{verbatim}
ar1  ma1  sma1  X1  X2  X3  X4
0.3190  -0.9528  0.1496  0.9893  0.1483  0.3536  0.3872
\end{verbatim}

s.e.  0.0801   0.0248  0.0777  0.4459  0.3962  0.5522  0.4120

\text{sigma}^2 \text{ estimated as } 148.7: \text{ log likelihood} = -702.61, \text{ aic} = 1421.21

$\text{degrees_of_freedom}$

[1] 172

$ttable$

\begin{verbatim}
Estimate  SE    t.value p.value
ar1  0.3190 0.0801  3.9822  0.0001
ma1 -0.9528 0.0248 -38.4093  0.0000
sma1  0.1496 0.0777 1.9239  0.0560
X1  0.9893 0.4459  2.2190  0.0278
X2  0.1483 0.3962  0.3743  0.7086
X3  0.3536 0.5522  0.6403  0.5228
X4  0.3872 0.4120  0.9399  0.3486
\end{verbatim}
$AIC$
[1] 6.079756

$AICc$
[1] 6.095545

$BIC$
[1] 5.203926

TTEST comparison of D and predD

Time period  2017 JJASON (SW monsoon season – Post Monsoon season)
D = reported number of dengue occurrences, predD = SARIMA forecast for 2017.

> D <- c(7, 14, 24, 31, 46, 4)
> predD <- c(19, 20, 20, 32, 17, 18)
> myD <- data.frame(group = rep(c("D", "predD"), each=6), value = c(D, predD))
> res2 <- t.test(D, predD, var.equal = TRUE)
> res2

  Two Sample t-test

  data:  D and predD
  t = 0, df = 10, p-value = 1

  95 percent confidence interval:
   -15.32942 15.32942

  sample estimates:
  mean of x mean of y
   21       21

Means for x and y series are identical => NOT significantly different.

NON-PARAMETRIC TEST (INDEPENDENT TWO SAMPLES) =>> WILCOXON’S TEST in R

> res3 <- wilcox.test(D, predD, paired= FALSE, exact= FALSE)
> res3

  Wilcoxon rank sum test with continuity correction

  data:  D and predD2
  W = 16, p-value = 0.8099

  Observed p-value is >0.05. Hence, NOT significant.

Thus, D and predD data sets follow identical distributions and are NOT significantly different.
Entomological surveillance and management of environment for prevention and control of dengue: an assessment

Amarpal Singh Bhadauriya, Pankaj U. Ramteke

Abstract

Dengue is one of the important public health problems in the world including India. Approximately four billion people are living at risk of dengue infection. Due to rapidly changing environment, dengue has dramatically expanded over the past decade worldwide. Due to unavailability of vaccine and proper treatment, it is very important to reduce the risk of dengue infection by using different vector control methods such as environment management, etc. Various studies have suggested that environmental management could be an important strategy to reduce the incidence of dengue. With the help of two important Aedes indices such as breteau index and pupal index, we can predict future outbreaks. These two important parameters could be used to know the geographical distribution of vectors and monitoring and evaluation of control programmes. This review aims to integrate data on the importance of environment management and entomological surveillance in dengue control activities. Data were collected from various sources such as WHOLIS, Scopus, Science Direct, Ovid, Google Scholar, POPLINE, etc.

Keywords: Aedes aegypti; environmental management; entomological surveillance; dengue

Introduction

Dengue is an arboviral infection, transmitted by the bite of infected female Aedes mosquitoes (1), particularly Aedes aegypti and Aedes albopictus. Dengue is contemplated as the most speedily spreading mosquito-borne disease worldwide (2). Approximately 128 countries worldwide are affected with dengue and dengue haemorrhagic fever with 50–100 million cases per year (3). Dengue and DHF is endemic in more than 100 countries including India. A recent study showed that 390 million dengue infections occur every year out of which approximately 96 million shows clinical manifestation (3). Several risk factors are responsible for rapid spread of dengue throughout tropics such as socioeconomic condition, environmental factors such as rainfall, temperature, relative humidity, rapid urbanization and

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quality of vector control measures taken in the areas. Integrated vector management is an important approach to curtail dengue transmission.

Climatic factors play an important role in dengue transmission and also affect life cycle of the vector. The rate of development of larva and pupa increases with increasing temperature, and emergence of adults as well as the biting rate of vectors also increase (1). The extrinsic incubation period (EIP) is one of the important factors in dengue transmission dynamics. It is the viral incubation period between the time when a mosquito draws a viremic blood meal and the time when that mosquito becomes infectious. Since the 1900s, the EIP has been recognized as an important factor in dengue transmission dynamics (4). EIP will be shortened with increase in temperature, which increases the biting frequency of the vector (5). Several studies have revealed that climate change can play an important role in epidemiology of dengue fever, because increased temperature and rainfall could increase viral transmission and this could also lead to the geographical expansion of the vector species of mosquito (4,6,7,8,9).

Environmental management approach is one of the most important strategies for dengue prevention and control. It involves the change of the environment in order to curtail or minimize vector density and human contact with the vector-pathogen by different methods such as source reduction, antilarval and anti-adult activities. According to WHO’s definition (10) environmental management can be categorized as (i) environmental modification; (ii) environmental manipulation; and (iii) changes to human habitation. Environmental management imply the improvement of water supply and water storage system among the community (11), mosquito-proofing of water-storage containers (12), solid waste management, community clean-up (13) and modification of building structures. Environmental management helps in understanding the ecology and population dynamics of the vector as well as the epidemiology of mosquito-borne diseases.

Entomological surveillance also plays a crucial role to find out the extent of prevalence of dengue vectors (5,14). Various entomological parameters such as house index (percent house positivity), container index (percentage of water containers positive for Aedes breeding), breteau index (number of positive containers for Ae. aegypti per 100 houses) and pupal index are usually used to identify the high-risk area. Pupal index provides information regarding vector density. Adult mosquito surveillance will also help in finding out the high-density areas.

**Importance of this review**

Due the absence of any specific treatment for dengue fever, prevention strategies specifically environmental, breeding source management and entomological surveillance have played an important role to curtail dengue transmission (15). Environmental management is considered as one of the most important components in dengue prevention and control. Many researchers have conducted studies on the role of environmental management in dengue control which is considerably relevant to reduce dengue transmission in the community. One systematic
review integrates information on one type of environmental control that examined changes in human behaviour, however combination of this method with chemical and biological methods still are not able to reduce dengue incidence (16). Entomological surveillance is an important tool for the detection of endemicity of dengue incidence by analysing various entomological parameters such as adult vector density (PMHD) and various indices such as house index, container index, pupal index and breteau index.

Entomological surveillance and environmental management interventions have been known to be effective strategies for the prevention and control of dengue incidences. In this comprehensive systematic review, we will amalgamate the information from various sources to know the overall effect of entomological surveillance and environmental management on the incidence of dengue. This study aims to systematically evaluate the effect of environmental management and entomological surveillance to prevent and control dengue incidences.

**Methodology**

For this review article we have searched Medline (PubMed), Cochrane Central, WHOLIS, Scopus, Science Direct, Ovid, Google Scholar, POPLINE, databases for articles published up to 2019. The main search terms included environment management, entomological surveillance importance, vector ecology, epidemiology of dengue fever, mortality, cost of illness, climate change, seasonal variation, moisture, population density, population growth, migration, housing, water, sanitation, water storage, rainfall, behaviour, control, service, prevention, environment, ecology, economic burden of dengue in India. Some information was also collected from the Centers of Disease Control (CDC), Pan American Health Organization (PAHO) and World Health Organization (WHO). We will be looking specifically at these three important interventions: (i) environmental modification: involves physical transformations to chop back vector larval habitat; (ii) environmental manipulation: temporary changes to vector habitats that involves the management of essential containers; (iii) entomological surveillance: evaluate various parameters.

**Result and discussion**

This review concluded that for the past few decades, dengue research has focused more on effects of climatic factors and importance of entomological surveillance for the prevention and control of dengue dynamics. However, sufficient data are not available for this kind of research in the Asia-Pacific region, where dengue is endemic in many countries. Previously, *Ae. aegypti* was most prevalent in urban areas but due to climatic change this vector expands his geographical distribution in rural areas too. Several studies suggested that *Ae. aegypti* flourish in both rural and urban areas (17,18,19) while *Ae. albopictus* in rural areas (20,21). Rapid urbanization is one of the important factors that brings people closer together and makes it easier for a mosquito to infect several people in a short time (22,23). One study
suggested that there is no significant relationship between the levels of urbanization in the incidence of dengue fever (24).

Among environmental factors, temperature plays an important role in dengue transmission. Srinivasa et al. (2017) (25) found that lower temperature ranges (\(\sim 17-18 \, ^\circ C\)) and high temperatures (\(\sim 35 \, ^\circ C\)) decrease the risk of dengue infection. Various other studies have examined the association between climate and dengue transmission (26,27). Johansson et al. (2009) (27) reported a positive association between short-term interannual variations in temperature, precipitation and dengue incidence. They also observed that this positive association varied spatially and was associated with differences in local climate as the effect of temperature on dengue incidence was highest in cool and wet mountainous areas, while the effect of precipitation was greatest in the hot and dry southwestern coastal region. A positive correlation with rainfall and dengue incidence was observed in a study conducted in Delhi, India. Increased rainfall resulted in multiplication of breeding sites, which increases the dengue infection with increasing adult density (4). Das et al. (2014) (28) found that weather parameters such as temperature, relative humidity and rainfall were positively correlated with the dengue vector density. They also found that the number of rainy days rather than the total rainfall was positively correlated with the larval density, which ultimately correlate with dengue transmission.

It is concluded from various studies that environment management practices such as environmental modification, environmental manipulation and changes to human behaviour are the most important practices which can reduce the possibility of dengue infection (15). The principle involved either the destruction of immature stages such as larvae/pupae or disruption of the mosquito immature life cycle. These can be effectively achieved through a public participation guided by health authorities in motivating the public to actively participate in vector control activities such as source reduction/antilarval activities. Environmental management provides an easily adaptable technique through which we can control dengue by using a wide variety of actions in an integrated fashion. Some studies revealed that residents are concerned about mosquitoes because of the “pest” factor and those concerns would be sufficient to motivate a certain level of behaviour change among the community (29). For control of Ae. aegypti in an environmental management framework, support and participation of the individuals and community are important (1). Water storage practice is a big problem in areas where water supply is irregular. In this situation, residents have to conduct cleanliness campaigns, dry day celebrations on a weekly basis to curtail mosquito breeding. A broader environmental management approach can also play an important role in reducing cases of dengue with greater intersectoral collaboration through advocacy. Singh and Taylor-Robinson (2017) (30) concluded that improvement in environmental health and management of vector habitats can reduce dengue incidences. Some studies conducted in India show that vector density increases during monsoon and post-monsoon seasons due to increased breeding habitat for Aedes mosquito (31,32).

Entomological surveillance is an important tool that can be used to determine changes in the geographical distribution and density of the dengue vector, evaluate control programmes
implemented by health authorities, obtain relative measurements of the vector population over time and space and facilitate appropriate and timely decisions regarding interventions. It can be used to identify any impending dengue outbreak in a particular area. It can also be used to change the outbreak alert level, the launch of a vaccination campaign, the initiation of vector control, the use of a different insecticide, susceptibility status of vector against insecticides that are being used for the control of vector species of mosquito. Various entomological indices such as house index/premise index, container index, breteau index and pupal index can be used to predict future dengue outbreaks. The breteau index is considered as the most important parameter because it can establish a relationship between positive containers and houses, i.e. it gives us information about positive containers as well as the house positivity rate. Pupal index is also an important parameter because it can be used to determine adult density and the relative proportion of the adult Aedes vector population comeuppance to each kind of container (33). One study indicates that increased breeding habitat increases the reproductive fitness of Aedes species by healthy pupal development (34). Adult mosquito density can be evaluated by many techniques. The torch and aspirator method is one of the easiest methods to collect adult Aedes species but the density of Aedes mosquito is low compared to other mosquito species and is difficult to estimate (35).

One study conducted in India showed that regular entomological surveillance along with community participation and social mobilization reduces Aedes breeding habitat and adult density that will ultimately reduce dengue cases (36). Another study shows that an outbreak of dengue was effectively controlled through entomological surveillance by carrying out antilarval and anti-adult activities (37).

Socioeconomic conditions are an important factor for rapid transmission of dengue virus. In a low socioeconomic community, people are not that much aware about the vector breeding habitat, behaviour of vector, etc. In most of slum areas – due to irregular supply of water – people had to store water in large number of plastic containers for their daily need, which increases the breeding habitat for Aedes mosquito (38).

For the success of vector control programmes, it is important to characterize the density–risk association and conduct prospective longitudinal cohort studies to assess mosquito density, dengue incidence and severity of disease. We should also know the correlation between Aedes species, abundance and dengue virus transmission. A detailed understanding of the relationships between mosquito ecology and the epidemiology of the disease help in strengthening public health programmes.

**Conclusion**

It is concluded that dengue is a major public health problem worldwide including India. Due to unavailability of any specific treatment and vaccine for dengue fever, it is important to use various controlling strategies such as environment management, regular entomological surveillance. Regular entomological surveillance can be used to determine changes in
geographical distribution of vectors, for monitoring and evaluating control programmes, for obtaining relative measurements of the vector population over time, and for facilitating appropriate and timely decisions regarding interventions. It can also play an important role in decision-making such as early warning signal by using a different entomological parameter. Breteau index and pupal index are the most important entomological parameters. Breteau index can provide information on per cent of house positivity as well as container positivity while pupal index gives information regarding adult vector density. Environment management strategies such as source reduction, improved water supply, proper disposal of waste material, installing of mosquito-proof windows, doors, use of personal protective measures against mosquito, etc., can reduce the risk of dengue infection. Community awareness regarding the vector is important for the prevention and control of dengue infection. Areas that are in the process of urbanization need to be monitored for prevalence of dengue and its vector, and appropriate vector control measures may be implemented.

References


Surveillance of breeding sites of dengue vector following the floods in an urban area of Patna, Bihar, India

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Abstract

Dengue is a vector-borne communicable disease caused by dengue virus (DENV) of the Flavivirus genus and transmitted by female mosquito mainly Aedes aegypti and to a lesser extent Aedes albopictus. The disease is distributed in more than 100 countries and spreading rapidly to other countries. In India, all 28 states and 8 Union Territories are affected from this dreaded disease. The records of dengue were available from 2012 in Bihar. The severe disease outbreak was found during the post-flood period in the month of October 2019. The surveillance of vector was conducted in highly affected mohallas of Patna urban. The survey was conducted by house-to-house visits. The population covered was 8514 in 1373 houses. Out of 6388 containers checked for Aedes larvae, 476 were found positive. The house index was found in the range of 6–40, the container index of 1–51 and the breteau index of 6–11. A total of 175 containers were treated with 1 ppm temephos. The community was sensitized for source reduction in 1019 houses. All indices came down after the ninth day. This disease outbreak came under control due to proper surveillance with timely and effective control measures. The situation may reappear after the monsoon in the following year if surveillance is ignored. Year-wise longitudinal surveillance is required to combat further transmission of the disease.

Keywords: Surveillance; breeding sites; Aedes; vector-borne disease; DENV

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Introduction

Dengue is a mosquito-borne viral disease caused by dengue virus (DENV) of the Flavivirus genus from four different serotypes (DEN-1, DEN-2, DEN-3 and DEN-4). It is distributed in more than 100 countries. The heaviest burden of dengue is reported by countries of the Asia-Pacific region including India, with 1800 million people at risk of dengue infection (1). India has been placed in “Category A” in terms of dengue endemicity by World Health Organization, South-East Asia (WHO-SEA) because it is a major public health problem in rural areas including other SEA countries such as Bangladesh, Indonesia, Maldives, Myanmar, Sri Lanka, Thailand and Timor-Leste (2). Aedes mosquitoes find favourable breeding sites due to the population growth, unplanned urbanization and poor water management systems (3). The breeding sites are mainly domestic water storage containers in and around human dwellings (4). The disease is transmitted by female mosquito mainly Aedes aegypti and to a lesser extent, Aedes albopictus. It is a small, black mosquito with white stripes and approximately 5 mm in size. It takes 7–8 days to develop the virus in its body and transmit the disease. Ae. aegypti breeds in any type of containers or storage container having even a small quantity of water. Eggs of Ae. aegypti can live without water for more than a year. It bites repeatedly in day-time to human beings in domestic and peridomestic situations. It rests in domestic and peridomestic situations in the dark corners of houses on hanging objects such as cloths, umbrellas, etc. or under the furniture. It is widespread throughout the tropics with local variations in risk influenced by rainfall, temperature and unplanned rapid urbanization. All the 28 states and 8 Union Territories in India are endemic for this disease. A few decades ago, dengue fever had a predominant urban distribution. It is now also reported from peri-urban as well as rural areas (5,6). In 2010, an estimated 33 million cases occurred in India (7). Dengue disease burden and frequent outbreaks adversely affect the country’s economy and put stress on the health systems. In India, case detection, case management and vector control are the main strategies for prevention and control of dengue virus transmission (8). Dengue cases were reported from July 2019 and continued till December 2019 in Bihar. There were heavy rains from the last week of September to the first week of October 2019 causing flood-like situation in the Patna urban area due to water-logging in many mohallas. Cases of dengue fever were reported from simultaneously from several mohallas. The government agencies and stakeholders took immediate steps to combat the situation. Vector surveillance and control measures were taken in cross-sectional follow-up studies. House-to-house searches were made from the ground to the top roof to identify the possible Aedes-breeding objects/containers in each mohalla, common places, community halls, shops, garages, godowns, etc. Information, education and communication (IEC) campaigns were conducted simultaneously to sensitize the people to act at a personal level to make their surroundings free from breeding sources and avoid fresh water deposition/storage in the dwellings.
Materials and methods

This cross-sectional study was conducted to interrupt the transmission of dengue disease by surveillance of immature stages of *Aedes* mosquitoes and their control. The urban population of Patna district is 2,049,156 spread over an area of 99 sq.km at 25.5941° N, 85.1376° E. The cases of dengue fever started from July and continued till December 2019. Due to heavy rains, a flood-like situation appeared from the last week of September to the first week of October 2019. The entomological surveillance was started in 27 affected areas of 15 localities from 5 October to 16 October 2019 in different affected mohallas such as Kant factory (Gandhi Nagar Road), Malawi Pakri (Sector D and J), Kankarbagh (Lohiya Nagar, PC Colony, Ashok Nagar Road No.1, Chitragupta Nagar and Defence Colony), Gardani Bagh (Bapu Nagar), Patliputra (Nehru Nagar, Industrial Colony, New Patliputra colony), Bhoot Nath Road, Rajendra Nagar (Road No.1 and 5), Gulzarbagh (Akhilesh Nagar), Bahadurpur (Saipur), Digha (Ashiana nagar), Kadamkuan (Budhamurti, Jagat Narayan Road), Sandalpur (Biscoomn Colony), Sampatchak, Chhaju Bagh, Rampur (Bazar Samiti) (Fig. 1).

*Fig. 1. Study area for surveillance of Aedes mosquito-breeding sites*

The breeding sites of *Aedes* mosquito were searched in these areas by door-to-door visits searching in pots, coolers, vases, overhead tanks, tires, lawns, gardens, parks, garages, shops, roadside solid waste, etc. The container-wise data were pooled and calculated for the
indices such as container index (CI): No. of Aedes larvae positive containers x 100/No. of containers with water inspected; house index (HI): No. of houses positive for Aedes larvae x 100/No. of houses inspected; and breteau index (BI): Number of positive containers x 100/No. of houses inspected.

Larval control measures were taken using 1 ppm temephos to the positive containers such as overhead tanks, tires and other water-containers. IEC activities were conducted at the household level to sensitize people to decant the stored water kept for a longer period.

Results

Cases of dengue were reported from July 2019. The government agencies were alert to control the disease transmission and teams were formed for active vector surveillance considering the increase in number of Dengue fever cases from the last week of September 2019. A total number of 1669 cases were reported from 20 September to 21 October 2019. Date-wise prevalence of cases in the study areas from 20 September to 21 October 2019 showed an increase in cases following the flood (Fig. 2).

Fig. 2. Prevalence of dengue cases in Patna urban areas

The most affected mohallas were Agamkuan (258 cases), Musallahpur-Bazar Samiti (108 cases), Boring Road (137 cases) and Patna (Middle area 92 cases). Surveillance of Aedes-breeding sites was conducted in Patna Urban areas covering a population of 8514 persons in 1373 houses. Of these, 272 houses were found positive for the presence of Aedes larvae. A total of 6388 different containers, such as earthen pots, overhead tanks, flower pot, solid wastes, coolers, refrigerators, etc., were examined for Aedes larvae, of which 476 containers were found positive. The overall house index (HI) was found in the range of 6–40, container index (CI); 1–51 and breteau index (BI); 6–11 (Table 1, Fig. 3 and 4).
**Table 1.** Entomological surveillance in dengue-affected areas

<table>
<thead>
<tr>
<th>Date</th>
<th>Area/ Locality</th>
<th>Water Status</th>
<th>Pop</th>
<th>Houses CHK</th>
<th>CHK +VE</th>
<th>Containers CHK</th>
<th>CHK +VE</th>
<th>Action Taken</th>
<th>HI</th>
<th>CI</th>
<th>BI</th>
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<td>05-10-2019</td>
<td>Kanti Factory/ Gandhi Nagar Road</td>
<td>Submerged</td>
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<td>20</td>
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<td>27</td>
<td>40</td>
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<tr>
<td></td>
<td>Malahi Pakri/ Sector D&amp;J</td>
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<td>306</td>
<td>48</td>
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<td>34</td>
<td>12</td>
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<td>17</td>
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<tr>
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<td>Water Receded</td>
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<td>52</td>
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<td>12</td>
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<td>23</td>
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<tr>
<td>06-10-2019</td>
<td>Gardani Bagh/Bapu Bazaar</td>
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<td>8</td>
<td>161</td>
<td>8</td>
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<td>14</td>
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<td>Patliputra/ Nehru Nagar</td>
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<td>Bhoot Nath Road/</td>
<td>Water Receded</td>
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<td>15</td>
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<td>90</td>
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<td>127</td>
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<td>11-10-2019</td>
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<td>7</td>
<td>305</td>
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<td>20</td>
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### Surveillance of breeding sites of dengue vector following the floods in an urban area of Patna, Bihar, India

<table>
<thead>
<tr>
<th>Date</th>
<th>Area/Locality</th>
<th>Water Status</th>
<th>Pop</th>
<th>Houses CHK</th>
<th>CHK +VE</th>
<th>Containers CHK</th>
<th>CHK +VE</th>
<th>Action Taken</th>
<th>SR</th>
<th>TT</th>
<th>IEC</th>
<th>HI</th>
<th>CI</th>
<th>BI</th>
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<td>12-10-2019</td>
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<tr>
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<td>Digha/ Ashiana Nagar</td>
<td>Water Receded</td>
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<td>318</td>
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<td>Water Receded</td>
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<td>Saibpur/ Bahdpur</td>
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<td>51</td>
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<td>Kadam kuan / Jagat Narayan Road and</td>
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<td>6</td>
<td>315</td>
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<td>11</td>
<td>4</td>
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<tr>
<td></td>
<td>Budhmurti</td>
<td>Water Receded</td>
<td>488</td>
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<td>5</td>
<td>334</td>
<td>7</td>
<td>5</td>
<td>3</td>
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<td>6</td>
<td>1</td>
<td>8</td>
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<tr>
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<td>Sandalpur/ Biscoman Colony</td>
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<td>220</td>
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<td>76</td>
<td>5</td>
<td>5</td>
<td>5</td>
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<td>35</td>
<td>12</td>
<td>7</td>
<td>12</td>
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<tr>
<td></td>
<td>Sampat chak/</td>
<td>Water Receded</td>
<td>244</td>
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<td>5</td>
<td>295</td>
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<td>9</td>
<td>5</td>
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<tr>
<td>15-10-2019</td>
<td>Jakkanpur/ Machhli Gali</td>
<td>Water Receded</td>
<td>373</td>
<td>48</td>
<td>11</td>
<td>215</td>
<td>17</td>
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<td>5</td>
<td>40</td>
<td>23</td>
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<td>15-10-2019</td>
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<td>312</td>
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<td>117</td>
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<td>7</td>
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<td>3</td>
<td>6</td>
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<tr>
<td>16-10-2019</td>
<td>Kankar bagh / Defence Colony</td>
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<td>413</td>
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<td>487</td>
<td>17</td>
<td>12</td>
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<td>17</td>
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<td>16-10-2019</td>
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<td>103</td>
<td>11</td>
<td>8</td>
<td>3</td>
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<td>19</td>
<td>11</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>16-10-2019</td>
<td>Rampur/ Bazar Samiti</td>
<td>Water Receded</td>
<td>8514</td>
<td>1377</td>
<td>272</td>
<td>6388</td>
<td>476</td>
<td>343</td>
<td>175</td>
<td>1019</td>
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</table>

Abbreviation : Population (Pop), Checked (CHK), Source Reduction (SR), Temephos treatment (TT), Information education and communication (IEC), House index (HI), Container index (CI), Breteau index (BI).
Larval control measures by source reduction were conducted in 343 containers by decanting water, breaking abandoned plastic containers, etc. A total of 175 containers such as water coolers, flower pots and overhead tanks were treated with 1 ppm temephos larvicide. The community was sensitized for source reduction during house-to-house searches covering a population of 1019. The rainfall recorded in the month of September 2019 was 151 mm. It was 1.2 mm during the survey period (5–16 October 2019). The minimum and maximum temperatures recorded were 22 ºC and 34.5 ºC, respectively and the relative humidity was 100% as per reports of the state meteorological department. After heavy rains, fresh water deposition was found in open terrace, gardens where generally unused pots, discarded tires
and abandoned solid wastes. *Aedes* larvae were found in most of these water reservoirs/containers with more in discarded tires (19.4%) and overhead tanks (16.0%). The containers on the ground were already flooded during the flood and breedings were flushed away. A majority of water-holding containers were on the rooftop. In some places, water coolers were found outside the houses without any care to change the water in time. A majority of them were found positive with *Aedes* larvae (Table 2). The key breeding containers could be identified in this limited study period.

**Table 2. Different types of containers searched for *Aedes* larvae**

<table>
<thead>
<tr>
<th>Container type</th>
<th>No. of containers checked</th>
<th>No. of containers positive</th>
<th>% positive</th>
</tr>
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<tbody>
<tr>
<td>Overhead tanks</td>
<td>350</td>
<td>56</td>
<td>16.0</td>
</tr>
<tr>
<td>Plastic tanks</td>
<td>1034</td>
<td>80</td>
<td>7.7</td>
</tr>
<tr>
<td>Metallic tanks</td>
<td>967</td>
<td>87</td>
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<tr>
<td>Cement tanks</td>
<td>563</td>
<td>45</td>
<td>8.0</td>
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<tr>
<td>Tyres</td>
<td>371</td>
<td>72</td>
<td>19.4</td>
</tr>
<tr>
<td>Flower pot</td>
<td>1523</td>
<td>55</td>
<td>3.6</td>
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<tr>
<td>Earthen pot</td>
<td>723</td>
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<td>6.1</td>
</tr>
<tr>
<td>Others</td>
<td>857</td>
<td>37</td>
<td>4.3</td>
</tr>
<tr>
<td>Total</td>
<td>6388</td>
<td>476</td>
<td>7.5</td>
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</table>

The IEC campaign helped in mostly emptying of the mud pots and solid waste and scrubbing to remove/destroy the eggs of *Aedes* attached to the wet surface of the containers. The community was advised to expose the container to sunlight for at least 2 hours.

**Discussion**

After 1996, dengue outbreaks upsurged in India during different years, such as 50 222 cases and 242 deaths (2012), 75 808 cases and 193 deaths (2013), 40 571 cases and 137 deaths (2014) (9). The risk of dengue has increased due to rapid urbanization and deficient water management including improper water storage practices in urban, peri-urban and rural areas, leading to proliferation of mosquito-breeding sites. The cases increase in monsoon and the pattern is not uniformly distributed throughout the year (10). Dengue transmission in India is a continuing phenomenon and each year the cases have been increasing, such as 1771 (2015), 1912 (2016), 1854 (2017), 2142 (2018 provisional) and 6193 (2019 November) without any death (11). A total of 6524 cases of dengue were reported from across Bihar including 4808 in Patna till 9 December 2019. It was a four-fold rise in cases compared to 2018. A total of
Surveillance of breeding sites of dengue vector following the floods in an urban area of Patna, Bihar, India

984 cases of dengue were reported in Patna in 3 months (August–September 2019) by the Bihar State Health Society from 1518 cases across the state. As no vaccine is available so far, vector control is the only option to prevent outbreaks of dengue (12). In the urban areas, there are high-density of water storage receptacles that are suitable for breeding of Aedes mosquitoes (3). In these areas, small numbers of Aedes-breeding habitats exist even during the adverse climatic conditions during the months of the year and act as “key containers” such as coolers, tyres, refrigerators, etc. (13). These are region-specific for Aedes breeding (14). In Philippines, plastic and metal drums are the key containers (15). Pitchers, solid wastes such as cold drink bottles without caps kept besides the shop, plastic shoes left on roadsides, coconut shells, broken bottles were also found positive for larvae in this study. Even the steel pot lying on the wall having rain water was found with a large number of larvae. The potential abandoned containers like tires on roofs and on the ground were found positive with larvae of Aedes in multistoried buildings and apartments. In Australia, larvae were collected in roof gutters (16). Cement tanks and plastic containers were identified as major breeding habitats of Ae. aegypti in India (17,18). In Delhi urban areas, overhead tanks and curing tanks were identified as key containers of Aedes breeding (19). Commonly, dengue infection occurs during or after the rainy season as an outcome of increase in the vector population (20). The transmission of dengue disease can be classified as wet and dry based on the rainfall season as Aedes breeding and dengue cases are observed in Delhi during June–November, referred as the transmission season, whereas December–May is referred as the non-transmission season for dengue (19). In a study at the western zone of Delhi having persistent dengue endemicity in 28 localities, it was found that a proper intervention in the non-transmission season reduces the vector density and subsequently dengue cases during the transmission season (21). In the present study, after the antilarval (AL) work, the HI was reduced from 44% to 11%, the CI from 51% to 3% and the BI from 100 to 25.

During the dengue outbreak in Tirunelveli, Tamil Nadu, India (latitude 8º42’ N; longitude 77.042º E) after the AL work, the HI was reduced from 48.2% to 1.6%, the CI from 28.6% to 0.4% and the BI from 48.2 to 1.6 (22). A longitudinal study needs to be conducted in Bihar to understand the trend and pattern of disease transmission and to conduct appropriate larval control measures to prevent further outbreak of the disease.

**Conclusion**

Dengue fever is an emerging mosquito-borne disease in Bihar. The number of dengue cases is increasing every year starting from the monsoon season to the post-monsoon period since 2012 in Bihar, India. In the surveillance during the last outbreak in 2019, breeding sites were mostly found in containers lying on the upper areas up to the roof. The maximum number of Aedes-breeding sites in Patna urban area was identified from tires of unused vehicles, water coolers and small pots kept on rooftops and outside the house, which were at some height where the flood water did not reach.
The larval control measures were neglected before this study. The key observations on the control aspect were timely reported to the government agencies for proper management and control the potential outbreak situation in the city. Further studies are required during transmission and non-transmission seasons to prevent further outbreaks of dengue in Bihar, India.

References


Surveillance of *Aedes aegypti* (L.) at different airport/seaports in India

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**Abstract**

The *Aedes aegypti* mosquito vector was found to be prevalent in all the airports/seaports in India. This species was non-existent during earlier surveys at the International Airport at Santa Cruz (Bombay), Dum Dum (Calcutta) and Palam (Delhi), which were kept free from *Ae. aegypti* by implementing vigorous anti-mosquito measures, although cities were not free from this species. Under the WHO, International Health Regulations (IHR 2005), all international airports and seaports are “kept free from all types of mosquitoes for a distance of 400 meters around the perimeter of ports”. Under IHR, *Ae. aegypti* index: “the ratio expressed as percentage between the number of houses in a limited well-defined area on the premises of which actual breeding of *Ae. aegypti* was found and the total number of houses examined in that area.” This index is kept at zero at all ports. Being hygroscopic, the *Ae. aegypti* depicted a phenomenon of “annual pulsation”. It tends to move to “mother foci” in the central parts of cities/airport/seaports which are humid during the dry season and spreads out during the wet season. The information proved to be of immense value for implementation of IHR and in delimiting areas that were prone to epidemics of dengue fever (DF)/dengue haemorrhagic fever (DHF). The vector population with high indices provided useful baseline information for developing control strategies in all airport/seaports. The container index ranged from 1.6% to 86.7% for *Ae. aegypti* at the Delhi Airport, while it was between 5.7% and 11.2% at Vizag and 23.4% and 30.7% at Cochin. The breteau index ranged between 1.8% and 33.3% for *Ae. aegypti* at Delhi and the highest breteau index (185.7) was reported at the Old Airport of Chennai.

**Keywords:** Surveillance; Aedes; airports; seaports; IHR.

**Introduction**

Dengue is one of the most rapidly spreading vector-borne viral disease globally, with an increasing number of areas at risk. It is caused by the dengue virus (DENV, 1–4 serotypes), which is one of the most important arboviruses in tropical and subtropical regions. Other *Aedes*-borne diseases are present in India, including chikungunya fever and Zika. The
epidemics of dengue in India have become more frequent in all the states. Earlier, the disease was restricted to urban areas, and has quickly spread to rural settings including northeastern states, i.e. Assam, Arunachal Pradesh, Tripura, Nagaland, Manipur, Meghalaya and Mizoram, where dengue was historically non-existent. The first outbreak of Aedes-borne disease was reported in 1824–25 at Calcutta, Madras and Gujarat (1-2). Dengue epidemics were reported again in Calcutta and Kanpur in 1847. The disease posed serious problems in 1870–73 from Bombay, Pune and Kolkata, Ganges valley, Ludhiana and Kerala coast from Calicut to Quilon, Madras. The disease was epidemic once again during 1901–07 in Calcutta, Ganges areas. Epidemics were reported during 1912–16 in Meerut in north India, 1950–60 in the Gangetic plain 48 km north of Calcutta, Kolaba district, Bombay state during 1960–70. Epidemics of dengue haemorrhagic fever (DHF) were reported in Calcutta in 1963; Vizag, Madras, Vellore in 1964; Nagpur city in 1965; Ajmer in 1967, 1970–80.

Sporadic cases in the country were reported throughout the decade of 1970–80; Delhi 1970, outbreak of dengue in Trichur district of Kerala in 1974, outbreak in Bewar town in 1976, worst outbreak of dengue in 1996 with more than 400 deaths in Delhi. The past few decades witnessed an increase in Aedes-borne diseases that have caused a huge economic burden in addition to loss of precious lives (3-5).

This period has also witnessed a boom in the vector population and their accelerated dispersal including airports/seaports. Primarily an urban problem, dengue has extended to semi-urban, rural and semi-arid regions of the country due to the spread of two vector species Aedes aegypti and Aedes albopictus. Ae. aegypti is prevalent in western, northern, Indo-Gangetic and Eastern plains, the Assam valley and coastal areas of Odisha. This species is non-existent in the Himalayan region. In the north-central highlands, the vector population showed low-to-moderate prevalence, while in south-central highlands, the population was high only in the valleys. The eastern plateau, including the Eastern Ghats, is comparatively free of the vector except large towns in Mahanadi basin. The Satpura ranges of the North Deccan were also found to be free from Ae. aegypti (1967) (6).

Dengue epidemics have been known to occur over the past two centuries in tropical and subtropical areas of the world. However, the role of the mosquito Ae. aegypti as the vector of this arbovirus has been known only during the past 70 years.

The entomological surveillance of Ae. aegypti and simultaneous appropriate interventions in key containers during the non-transmission months (December–May) would have any impact on breeding of Aedes and dengue cases during the transmission months (June–November). The impact of the surveillance and intervention measures undertaken during non-transmission months were assessed by entomological indicators, namely the container index (CI), house index (HI), pupal index (PI) and breteau index (BI) (7).

Ae. aegypti is highly domesticated, anthropophilic, multiple feeding, triggered by discordant and interrupted feeding behaviour resulting in heterogeneous pool of viraemia, which has rendered this species as the most efficient vector.
Material and methods

The study covered different airports/seaports, viz. Delhi, Chennai, Kolkata, Tiruchirapalli, Vizag, Kandla, Tuticorin and Kochi. Entomological surveillance of Aedes mosquito has been standardized on different indices based on the simple determination of presence or absence of Aedes larvae either in each container or somewhat in each house (7,8). The house index (HI, percentage of houses positive for larvae), container index (CI, percentage of containers positive for larvae), breteau index (BI, number of positive containers per 100 houses), and premises index were calculated. All non-hermetically closed containers containing any volume of water were considered as potential breeding sites. All water-holding containers were examined. Either the name/type of the containers (namely, tire, cooler, fire extinguisher bucket, etc.) or construction materials (namely, cement tank, earthen pots and plastic container) were classified as the breeding sites. The number of surveyed premises, houses, positive containers (with Aedes pupae or larvae), and houses with ≥1 positive container were recorded in the study. The conventional methods of Aedes survey as adopted by the National Vector Borne Disease Control Programme (NVBDCP) for outbreak studies were followed. As the scope of the study was limited to determining the distribution of the species, the information was collected on the following aspects: (i) occurrence (as evinced by indigenous breeding); and (ii) intensity of infestation (No. of houses found positive per ward) house (premises) index: No. of houses positive for Aedes larvae/No. of houses examined x 100 prevalence of Ae. aegypti.

Results

Regular Aedes larval surveys at different airport/seaports and outbreak surveys provide Aedes surveillance data in the country. The breeding infestation pattern of Aedes breeding is indicated by entomological data from surveys. The baseline Ae. aegypti distribution is available for different states in the country (Fig. 1). But these Aedes surveys also showed the distribution of vector species particularly in the northeastern states and Jammu and Kashmir, which were earlier free from Aedes-borne diseases. The information on vector surveillance at airport/seaport is not available. The vector surveillance at Delhi Airport revealed that container index was very high in all the 3 years of survey (Table 1–3 & Fig. 2). Similarly, the vector container indices were very high at Kandla, Kolkata, Chennai, Tuticorin and Tiruchirapalli. The house index was highest (39.6%) at Kandla. The breteau index was very high (185.7) at the Old Airport of Chennai. The Vizag Airport also reported a very high premise index during the survey in 2013 (Table 3). The Kochi Port revealed a very high container index during 2009. It clearly indicates that the vector population survives in these areas with a high reproductive potential in the absence of anti-larval measures.
Table 1. Vector surveillance at IGI Airport, Delhi

<table>
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<tr>
<th>S. No.</th>
<th>Date</th>
<th>Locality</th>
<th>No. of places checked</th>
<th>No. of places positive</th>
<th>Genus</th>
<th>No. of premises checked</th>
<th>No. of premises positive</th>
<th>No. of container checked</th>
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</table>
Surveillance of *Aedes aegypti* (L.) at different airport/seaports in India

**Fig. 1.** Localities where *Aedes aegypti* has been found in India

- *Aedes aegypti* was distributed all over India as per Barraud’s faunatic survey 1934.
- This was subsequently also confirmed by follow-up surveys.
- Dengue virus distribution in India might have documented very late.

![Map showing localities where Aedes aegypti has been found in India](image)

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**Table 2.** *Aedes* survey in PHO/APHO, Kandla, Kolkata, Chennai, Tuticorin and Tiruchirapalli, 2008

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Location</th>
<th>HI</th>
<th>CI</th>
<th>BI</th>
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<td>1</td>
<td>Engineering Workshop, Kandla</td>
<td>44.4</td>
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<td>2</td>
<td>Water Workshop, Kandla</td>
<td>11.1</td>
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<td>3</td>
<td>PHO Staff Colony-I, Kandla</td>
<td>28.6</td>
<td>37.5</td>
<td>42.9</td>
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<td>4</td>
<td>IFFCO, Government Building, Kandla</td>
<td>21.4</td>
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<td>5</td>
<td>Seaport Building outside, Kandla</td>
<td>11.1</td>
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<td>6</td>
<td>Port Trust Hospital, Kandla</td>
<td>12.5</td>
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<tr>
<td>7</td>
<td>PHO Staff Colony-II, Kandla</td>
<td>21.4</td>
<td>14.3</td>
<td>28.6</td>
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<td>8</td>
<td>Fisherman Colony, Kandla</td>
<td>39.6</td>
<td>37</td>
<td>70.8</td>
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<td>9</td>
<td>Port Trust Building inside, Kandla</td>
<td>35.6</td>
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<td>10</td>
<td>Kandla Port Trust (Jattly Office)</td>
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<td>11</td>
<td>Netaji Subhas Docks, PHO</td>
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<td>12</td>
<td>Airport, Kolkata</td>
<td>27.9</td>
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<td>13</td>
<td>PHO, Tuticorin</td>
<td>6.2</td>
<td>15</td>
<td>18.8</td>
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<td>14</td>
<td>Old Airport, Chennai</td>
<td>35.7</td>
<td>46.4</td>
<td>185.7</td>
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<td>15</td>
<td>Airport, Tiruchirapalli</td>
<td>3.1</td>
<td>37.8</td>
<td>53.1</td>
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Table 3. Vector surveillance at Vizag Port, 2013

<table>
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<tr>
<th>S. No.</th>
<th>Location</th>
<th>Premise index (PI)</th>
<th>Container index (CI)</th>
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<td>Airport</td>
<td>33.3</td>
<td>11.29</td>
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<td>2</td>
<td>Seaport</td>
<td>43.7</td>
<td>8.03</td>
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<td>3</td>
<td>Adjoining areas (AP)</td>
<td>21.4</td>
<td>7.31</td>
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<td>4</td>
<td>Kotavidi</td>
<td>24.19</td>
<td>7.69</td>
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<td>Adjoining areas (SP)</td>
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<td>6</td>
<td>Adjoining areas (SP)</td>
<td>30</td>
<td>14.7</td>
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<td>7</td>
<td>East India Petroleum and Oil Work area</td>
<td>20</td>
<td>5.74</td>
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</table>

**Fig 2. Aedes survey in Cochin Port, 2009**

Discussion

Dengue is on the rise in the past few years with epidemics reported in different states including airport/seaports. The breeding was detected in different containers at all airport/seaports including containers in residential colonies. The empty paint containers for ships were a potential breeding source at Vizag Port. During the survey it was observed that all airports/seaports have limited infrastructural facilities and inadequate staff for vector control, which results in the expansion of *Aedes* mosquito vectors during the transmission season. There are multiple agencies at airports/seaports, which contribute to creating mosquitogenic conditions; thus regular capacity building is required for prevention and control of disease vectors.
The *Ae. aegypti* population depicted a terrain-bound phenomenon of “annual pulsation”. The population showed a definite reduction during the dry season and expansion during the wet season. Both drought conditions and high rainfall were found to encourage a high build-up of *Ae. aegypti* population. In the former case, water storage practices were followed due to water scarcity, and in the latter case, abundant availability of secondary foci in domestic and peri-domestic areas promoted the growth of high vector populations. A complete absence of breeding was observed in extra-domestic habitats. This showed continued dependence of the species on humans for food and shelter and complete absence of any sign of ecological adaptation towards feral situation which increases its epidemiological potential (9-11).

During vector surveillance, it was observed that all the entomological indices were above the critical level and the IHR (2005) recommendations were not implemented. Therefore, seaport authorities need to give more attention to implementing IHR (2005) to counter the mosquito-breeding problem. Thus, a careful invigilation of the international airports and seaports should be done by vector control experts to identify any accidental and deliberate import of disease vectors and implement bio-security and quarantine measures to prevent potential international health risks. Regular surveillance should be carried out before and after the monsoon season (12).

**Utility of observations**

WHO IHR (2005) clearly mentioned that every conveyance leaving a point-of-entry situated in an area where vector control is recommended should be disinfected and kept free of vectors. It also mentioned that State Parties shall establish programmes to control vectors that may transport an infectious agent that constitutes a public health risk to a minimum distance of 400 meters from those areas of point-of-entry facilities that are used for operations involving travellers, conveyances, containers, cargo and postal parcels, with extension of the minimum distance if vectors with a greater range are present. The vector surveillance data collected will help in delimiting the areas prone to *Aedes*-borne epidemics at airports/seaports.

**References**


Dengue in Chennai: a retrospective study

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Abstract

Chennai city is encountering dengue for the past two decades with more cases during certain years. The exact trend of the disease is not clear and whether the disease is endemic or epidemic could not be ascertained. This information is essential for the vector control managers to implement control strategies at appropriate time to prevent or reduce the incidence. In this study, the 21 years’ dengue data pertaining to Chennai city available with the Vector Control Department of Greater Chennai Corporation was analysed using a formula, long-term mean (LTM) and the impact of daylight duration on the incidence of dengue. The results indicated that this disease has a specific trend and the prevalence exhibits epidemic proportions during certain years.

Keywords: Dengue; Chennai; LTM; daylight; temperature; rainfall; epidemic proportion.

Introduction

Dengue is an emerging vector-borne disease. Half of the world’s population lives in dengue-endemic regions, particularly in South-East Asia, the Pacific region, and the Americas. About 80% of people infected with dengue virus are asymptomatic. Approximately 5% of people diagnosed with dengue have more severe illness and 1% may develop severe life-threatening infections. Chennai is located at 13° 04N and 80° 17E on the southeast coast of India and in the northeast corner of Tamil Nadu state abutting the Bay of Bengal. The temperature recorded here is between 15.8 °C and 44 °C. The relative humidity ranges between 61% and 80%. The average annual rainfall is about 1300 mm. The city gets most of its rainfall from the northeastern monsoon during October to mid-December. A fair amount of rainfall is also received from southwest monsoon usually called as summer rain.

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Dengue history

The dengue disease has a long history before evolving to be a life-threatening epidemic today. In 992 CE, a Chinese medical encyclopaedia described the fever as “water poison” as it is associated with flying insects (1). In 1780, in India the first epidemic of dengue was recorded in Madras (2). The first widespread use of term “Dengue” started in Cuba in 1826 after an epidemic (3). Dengue virus was isolated as a “virus” in 1943 during an epidemic in Nagasaki, Japan (4). In 1944, the virus was isolated in India from Calcutta (now Kolkata) from US soldiers (5). The first major epidemic occurred during 1953 in the Philippines and quickly spread across the globe (6). In 1996, the first major epidemic in India occurred in Delhi and Lucknow and spread across the country (7,8). In 1997, WHO divided dengue into undifferentiated fever, dengue fever and dengue haemorrhagic fever (9). In 2003, four serotypes of dengue were detected in Delhi (7). In 2009, WHO divided dengue fever into two groups: uncomplicated and severe when it is haemorrhagic. In 2013, a total of 78 808 cases were reported in India, the maximum in the past seven years (9). In 2015, dengue was reported from 30 states and Union Territories of India and seven states reported dengue early in January (10).

Definition of epidemics

Before comprehending the trends of dengue epidemic in Chennai, we need to understand the definition of epidemic and disease outbreaks from the public health perspective. We use an arbitrary definition of “epidemic”. From the public health point of view, a single year where seroprevalence increases by at least 10% is considered to be an epidemic year. Ten per cent was selected because any disease involving that proportion of the population would be considered an epidemic. This level of transmission would result in just slightly more than 1% of the population being infected during the peak of the epidemic, a minimum value that has been suggested as sufficient for the detection of transmission. An outbreak is defined as the sudden occurrence of a disease in a community, which has never experienced the disease before or when cases of that disease occur in numbers greater than expected in a defined area. Whereas an epidemic is defined as an occurrence of a group of illnesses of similar nature and derived from a common source, in excess of what would be normally expected in a community or region. Some diseases can remain active in a given area for years and years. A disease is described as endemic when it is habitually present within a given geographical area. Although dengue, which is spread by mosquitoes, is endemic in more than 100 countries, it is not considered a pandemic. The point to consider here is that the dengue cases are not from a common source as the vector mosquitoes are not capable of flying beyond a few hundred metres. So the cases in each country are from a different source.

An outbreak is defined as “the sudden occurrence of a disease in a community, which has never experienced the disease before” or “when cases of that disease occur in numbers greater than expected in a defined area”. Therefore, it is clear that in Chennai, dengue is neither a sudden occurrence nor a never experienced disease in the community. Chennai city
has experienced frequent dengue outbreaks for the past two decades. This study attempts to assess the prevalence of dengue in Chennai and its trend. This article aims to perform temporal decadal analysis of dengue incidence in the Chennai Metropolitan area (1999–2019); to identify the temporal incidences that have exceeded the monthly threshold using long-term mean (LTM) analysis; to compute disease trends in years: epidemic and non-epidemic trends; and to analyse the influence of daylight temperature on dengue vector epidemiology.

**Materials and methods**

The dengue data available with the Vector Control Department of Chennai City Corporation, the municipal body of the state government of Tamil Nadu were collected, and daily duration of daylight obtained from the Meteorological Department was compiled and used for analysis. A simple formula has been adapted to determine the threshold of cases and the number of occasions the cases have exceeded the threshold. We tried to perform a temporal decadal analysis of dengue incidence in Chennai for two decades, by computing the yearly aggregate disease incidences, month-on-month (Tables 1 and 2).

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Table 2. Monthly incidence of dengue in Chennai city from 2010 to 2019

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When the monthly incidence is plotted on a graph the incidence shows extreme spikes and a definite trend of the disease could not be assessed precisely (Figs. 1 and 2).

Fig. 1. Prevalence of dengue cases in Chennai city for 11 years (1999–2009)
Hence to gain insights into possible disease trends, the LTM analysis method was adopted. The LTM analysis method was most preferred to compute temporal changes of a frequency distribution (11). The LTM method is the sum of all the individual dengue cases (Σ cases) in the primary data, divided by the number of months taken for the year of study (2009–2019) (Σ Months). The mean threshold computed using this approach gives a precise measure of central tendency by using specific value of each epidemiological score to compute mean. The mean computational statistics also has statistical properties of being scaled as an interval or a ratio (12). The formula for computing LTM thresholds using means is:

**Formulae**

\[
LTM = \frac{\sum \text{cases}}{\sum \text{months}}
\]

\[
LTM = \frac{\sum \text{Cases 7168}}{\sum \text{Months 252}} = 28.44
\]

After determining the LTM as 28.44, it has been decided to take into consideration the number of months the incidence had exceeded the LTM (28.44) in a given year, which is shown in red colour font for easy identification (Table 3). It would be appropriate to consider it an epidemic year whenever the incidence exceeds the LTM for 3 months and more.
Table 3. Number of months the incidence has exceeded the LTM during 1999–2019

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|       | 5    | 6    | 6    | 2    | 1    | 2    | 0    | 5    | 3    | 7    |

Values in red denote months exceeding LTM
Based on the LTM data in Table 3, a graph was constructed to depict the prevalence of dengue in Chennai (Fig. 3). Year-wise disease trends, the total incidences of a year that exceeded the mean threshold values, were visualized. It was observed that in the past 21 years, the epidemics have occurred only during 11 years.

**Fig. 3.** Prevalence of dengue in Chennai city (1999–2019)

The LTM analysis data was also used to evolve the monthly trends on dengue vector epidemiology. The 21-year data were converted into monthly average and a graph built on the data indicates a clear trend of dengue in Chennai (Fig. 4).

**Fig. 4.** Monthly trend of dengue in Chennai city
Impact of the duration of daylight on the incidence of dengue

An attempt was made to study the impact of daylight on the incidence of dengue as the vector mosquito, *Aedes aegypti* is essentially a day biter with peak biting during certain times of the day well within the daylight period. The monthly sunrise and sunset data for the period from 2000 to 2017 were obtained from the Meteorological Department and the extent of average daylight period was calculated. Fig. 5 with the above data indicates that a period of 11–12 hours of daylight (longer day and shorter nights) enables the vector to bite more and more people and enhance the degree of transmission from the month of February up to August.

**Fig. 5.** Incidence of dengue in relation to the duration of daylight in Chennai city (2000–2017)

Discussion

Dengue cases are reported in Chennai with retrospective perspectives (13–16). Rainfall plays a considerable role in the outbreak of dengue and its transmission (14, 17, 18). Entomological indices, viz. house index, container index, pupae index and breteau index (19), play a vital role as they forecast the early warning signs of a possible outbreak of dengue, along with threshold values (20–22). Several studies were conducted earlier using larval indices. Conversely, in this article, the novel concept of LTM was used. Chennai is a water-starved city, and the temperature, relative humidity and rainfall play an important role with regard to the density of *Aedes* mosquitoes and in the transmission of dengue. From February to August, owing to the hot humid climate, people tend to store water inside houses, and *Aedes* breeds indoor and less cases of dengue are reported. With intermittent summer rains, the vector moves outdoors where more habitats are available for proliferation. The combined density of indoor and peri-domestic vector density increases the transmission potential...
attaining the peak from August to October. Due to slashing of heavy rains by the northeast monsoon in November (month of cyclones) peri-domestic breeding mostly occurring in trash gets washed off.

The temporal decadal analysis of dengue incidence proves that the disease has become perennial in Chennai. The disease incidence was not zero in any year and there was a specific pattern of occurrence with a peak in the month of October followed by a decline from the month of November up to February. After a lean period of 2 months (March and April) the cases again start to show an inclining trend from the month of May onwards. This proved that dengue disease spread varied over temporal time-scale (23). The temporal incidences exceeded the LTM thresholds only during 11 years. A visualization of epidemic and non-epidemic years helped us confirm the findings. From the public health perspective, the monthly and yearly dengue incidences that exceeded the threshold value can be declared as the season of disease outbreaks and the year of epidemic.

The study also identified the influence of climate of dengue vector epidemiology of Chennai. The following climatic factors may be contributing to the rise and sustenance of the disease in Chennai.

1. January–February: Cooler nights and hotter days; adult vector mosquitoes continue to survive and transmit the cases.

2. March–April: Rise in day-time and night-time temperatures; ideal time for developing the process of transmission; longer daytime enhances the man–vector contact.

3. May–June: Summer rain creates more suitable sites for vector breeding. The vector mosquito which hitherto preferred to breed indoors comes out and proliferates. High density and ambient temperature and humidity start rolling the transmission process.

4. July–August: Rise in the incidence leads to a peak.

5. September–October: Attains the peak in the month of October. Northeast monsoon sets in and intermittent and excessive rains wash away mosquito breeding to a large extent. A drop in the temperature prolongs the metamorphosis and the density declines.

6. November–December: No more peaks in the incidence; cases start to show a declining trend; longer nights and shorter days reduce the man–vector contact (day-biting mosquito) and the cycle continues.

The above trend indicates there is a strong influence of climatic variables on transmission potential of dengue vector and epidemiology. The seasonal variation in the number of cases strongly correlated with the daylight hours and monsoonal timelines (24). The present study
may be taken as a model to construct the trend of dengue in any geographical area. The LTM value, which is applicable to the study area should be taken as a key threshold figure, and any increase in its value should cause the alarm bells ringing and forewarn the vector control managers. This methodology may be applied to any other disease of local importance. This article shows the efficient usage of LTM in association with the weather parameters as a modelling study in Chennai with special reference to dengue.

References

Dengue in Chennai: a retrospective study


Community acceptance of temephos (1% sand granules formulation) and its susceptibility to Aedes spp. larvae in Myanmar, 2017

Nay Yi Yi Linn, a Badri Thapa, b# Aye Aye Myint, a San San Win, b Thaung Hlaing, c Thiha, a Aung Thi, a Nwe Ni Lin, a Aye Mon Mon Kyaw, d Myat Min Tun, d San Kyawt Khine, b Sithu Ye Naung, d Thiha Myint Soe, d Wint Phyo Than, d Zaw Lin e

a Vector Borne Disease Control Programme (VBDC), Department of Public Health, Ministry of Health and Sports, Myanmar

b World Health Organization, Myanmar

c Department of Public Health, Ministry of Health and Sports, Myanmar

d State/Region Vector Borne Disease Control Programme, Department of Public Health, Ministry of Health and Sports, Myanmar

e World Health Organization, South-East Asia Region

Abstract

Background

The Myanmar Dengue Prevention and Control Programme used temephos 1% sand granules formulation extensively in water-storage containers in 2017. This study aims to assess the knowledge on dengue cause, transmission and prevention, prevention practices, community acceptance of temephos, and its susceptibility to Aedes spp. larvae in Myanmar.

Methods

During January–June 2017, this community-based cross-sectional study was carried out in purposively selected six states/regions with high dengue cases in 2016. A structured questionnaire was administered to 120 respondents. Temephos susceptibility test was done as per the standard WHO guideline for Aedes spp. larvae collected from 60 breeding sites across the six states/regions.

Results

The participants had high knowledge of dengue cause and transmission – mosquito bite (99.2%), Aedes mosquito (94.0%) and day-time biting (95.8%). They also had high knowledge of prevention – covering water containers (97.5%), applying mosquito repellents (93.3%) and removal of standing water (93.2%). At the same time, misconceptions prevailed about transmission – personal contact (19.5%) and sexual intercourse (8.9%). Respondents mostly practiced dengue prevention using

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temephos as larvicide in water-storage tanks (95%) despite lower community acceptance of smell (35.1%) and turbidity (11.7%). Other prevention measures practiced were pest control (90%), environmental management (elimination of standing water 89%; cleaning bushes 90%), cover water containers (88%) and bed nets (88%). Temephos was 100% susceptible to Aedes spp. larvae in 24 hours of the assay.

**Conclusion**

The study showed that community knowledge of dengue cause, transmission and prevention was high. Most of the community used temephos – which was 100% susceptible to Aedes spp. larvae – despite the dislike for smell and turbidity. Continuous advocacy through a national programme is needed to motivate communities to use it. The efficacy of temephos needs to be monitored regularly.

**Keywords:** Dengue; knowledge; temephos; Myanmar.

**Introduction**

Sporadic cases of dengue have been reported since 1960 in Myanmar (1). In 1970, the first dengue outbreak occurred in Yangon with 1654 cases and 91 deaths, which later spread to other states/regions in 1974. In 2015, all states and regions in Myanmar reported dengue cases. The number of reported dengue cases increased from 1654 in 1970 to 43 845 in 2015. On the other hand, the case fatality rate (CFR) decreased from 5.50% in 1970 to 0.37% in 2015. Dengue cases increase in the rainy season with the peak in July; more than half of the dengue cases are found in rural areas; children under 15 years are mostly affected from dengue, especially the age group of 5–9 years; and all four serotypes of dengue virus are in circulation. Aedes (Stegomyia) aegypti is the primary vector (1).

The National Strategic Plan for Dengue Prevention and Control, 2016–2020 was developed, which envisaged to reduce dengue morbidity by at least 25% by 2020 and maintain the CFR to less than 1% (2). Various vector control strategies that aim at controlling Ae. aegypti are currently used to curb dengue cases and prevent outbreaks. These vector control interventions often use chemical or biological agents for the control of immature and adult mosquito stages, or environmental control methods that target mosquito breeding sites (3,4). In line with WHO, the Dengue Prevention and Control Programme has put enormous efforts to reduce the number of cases and deaths through chemical control – use of temephos sand granules for Aedes spp. larval control (5). But, the programme encountered many challenges – most importantly, temephos has an objectionable odour and causes water turbidity that could hinder the community use (6), and loss of granules during the process of cleaning water-storage containers (6). The programme currently uses environmental
manipulation, modification, biological and chemical measures – including temephos (7). The programme has used temephos 1% sand granules formulation (SDS Ramcides Cropscience Pvt. Ltd., India) extensively in 2016. This has considerably reduced entomological indices (8). However, no systematic study has probed community acceptance of the temephos sand granules formulation (SDS Ramcides Cropscience Pvt. Ltd., India) and assessed its sensitivity as a larviciding agent against the dengue vector. This study aims to assess various factors such as knowledge of dengue cause, transmission, prevention and practices, and community acceptance of temephos. The study findings could provide insights on community acceptance and sensitivity of temephos in Myanmar.

**Materials and methods**

**Study design**

This community-based cross-sectional study was carried out during January–June 2017 in purposively selected six states/regions (Yangon, Ayeyarwady, Rakhine, Mon, Kayin and Bago) with a high incidence of dengue.

**Study settings**

**General setting:** The Republic of the Union of Myanmar is one of the South-East Asian country which is administratively divided into 14 states/regions and Nay Pyi Taw Council Territory. There are 74 districts with 330 townships and the population is 51.5 million with nearly 70% residing in rural areas (1). The Ministry of Health and Sports provides clinical care services to the whole population through hospitals under the department of medical services, and the department of public health mainly focuses on health care in public health areas in urban and rural areas.

**Specific setting:** During January–June 2017, the Dengue Prevention and Control Programme conducted mass larviciding with temephos 1% sand granules in six states/regions (Yangon, Ayeyarwady, Rakhine, Mon, Kayin and Bago) (Table 1, Fig. 1). A total of 394,250 kg of temephos was used that covered 4.5 million population. All Union Territories, states/regions are endemic for dengue in Myanmar. These six states/regions contributed to 59% of cases and 73% of deaths in the country in 2016. Since 1964, dengue is a notifiable disease in Myanmar and it is mandatory for all public health institutions to notify dengue.
Table 1. Temephos distributed and population covered by states/region in 2017

<table>
<thead>
<tr>
<th>S. No.</th>
<th>States/regions</th>
<th>Population covered</th>
<th>Temephos in kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yangon</td>
<td>1 353 909</td>
<td>172 750</td>
</tr>
<tr>
<td>2</td>
<td>Ayeyarwady</td>
<td>1 279 098</td>
<td>102 500</td>
</tr>
<tr>
<td>3</td>
<td>Rakhine</td>
<td>45 248</td>
<td>25 000</td>
</tr>
<tr>
<td>4</td>
<td>Mon</td>
<td>280 573</td>
<td>32 750</td>
</tr>
<tr>
<td>5</td>
<td>Kayin</td>
<td>384 056</td>
<td>25 000</td>
</tr>
<tr>
<td>6</td>
<td>Bago</td>
<td>1 121 271</td>
<td>36 250</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>4 464 155</td>
<td>394 250</td>
</tr>
</tbody>
</table>

Fig. 1. The study states/region (Blue) and the study townships for larval collection and in-depth interviews (green)
Knowledge of cause, transmission and prevention, prevention practices and community acceptance of temephos

Five townships were selected from each state/region (a total of 30 townships) with highest number of cases and where the temephos was used during January–June 2017. Four respondents were selected randomly from each township (a total of 120 respondents) and an in-depth interview of each respondent was conducted using a structured interview questionnaire. The Vector Borne Disease Control Staff and the Basic Health Staff of each township were trained to conduct in-depth interviews based on the guidelines translated into Burmese. The guidelines and questionnaires were piloted and revised before their use for the study.

Temephos susceptibility test

Aedes spp. larva was collected from 60 breeding sites in 12 townships from the six states/regions based on purposive sampling. The susceptibility of larvae to standard temephos (Temeguard 1% SG, Ladda Company Ltd, Thailand) certified with 1% temephos was assessed using the standard WHO guideline (9). Each test used 25 late-third or early-fourth instars in plastic cups with 99 ml of mineral water and 1 ml insecticide solution at the required concentration. Three different concentrations in the range of activity of temephos (0, 0.5, 1.0 ppm) were used and 25 larvae per concentration with four replicates of 25 larvae were tested against each concentration. The 0 ppm was used as the control group. Tests were run at 27 ± 2 °C, and mortality was assessed after 0.5, 1, 2, 3, and 24 hours of insecticide exposure.

Data entry, analysis and statistics

In-depth interview questionnaires were analysed quantitatively and expressed in numbers and frequencies. The larval mortality rate was calculated and expressed in percentage and compared over different time-points. The following WHO criteria were used: susceptible when mortality was 98% or higher, possible resistant when mortality was between 80% and 97% (inclusive), and resistant when mortality was below 80% in 24 hours.

Ethics approval

Ethical approval for the study was taken from the University of Public Health, Myanmar. Permission for the study was sought from the Dengue Prevention and Control Programme.
Results

The present study was conducted to understand the community knowledge of dengue cause, transmission and prevention; prevention practices; and acceptance and susceptibility of Aedes spp. larvae to temephos 1% sand granules in the six states/regions. The results are presented in the following sections.

Demographic characteristics of the respondents

Of the 120 participants, 73 (61%) were males and 47 (39%) were females. Of these, 26 (22%) had primary, 63 (52%) had secondary and 31 (26%) had tertiary education. The most common occupation group was dependent (unemployed) (43%) followed by worker (19%), farmer (13%), health staff (8%), local administrator (8%) and others (9%).

Dengue knowledge of cause, transmission and prevention

Table 2 presents the dengue knowledge of cause, transmission and prevention in 120 participants among which in-depth interviews were conducted. The participants had high knowledge of dengue caused by mosquito bite (99%), mosquito biting time in the afternoon (96%), dengue caused by Aedes mosquito (94%), and that dengue is prevented by covering water containers (98%), applying mosquito repellents (93%), and removal of standing water (93%). However, misconceptions prevailed about transmission by personal contact (20%) and sexual intercourse (9%).

Dengue prevention practices

Table 3 presents the response of participants on various dengue prevention practices. Most respondents always covered water containers (65%) and used bed nets (66%). Some respondents usually called the pest controller (31%) and removed standing water (30%). Some respondents sometimes used spray (55%) and mosquito coil (53%), while window screens (52%) and fans (32%) were never used. Prevention practices when combined (sometimes to always) – most used prevention practices were: temephos (114, 95%), pest control (90%), environmental management (elimination of standing water 89%, cleaning bushes 90%), cover water containers (88%) and bed nets (88%).
### Table 2. Dengue knowledge of cause, transmission and prevention, Myanmar, 2017

<table>
<thead>
<tr>
<th>Knowledge of</th>
<th>Total</th>
<th>Yes</th>
<th>No</th>
<th>No response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td><strong>Cause</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caused by mosquito bite</td>
<td>120</td>
<td>119</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dengue mosquito feed/bite in the afternoon</td>
<td>120</td>
<td>115</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td><strong>Transmission</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flies transmit dengue fever</td>
<td>120</td>
<td>12</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>All types of mosquitoes transmit dengue fever</td>
<td>120</td>
<td>29</td>
<td>24</td>
<td>76</td>
</tr>
<tr>
<td>Aedes mosquito transmits dengue fever</td>
<td>120</td>
<td>110</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Person-to-person contact transmits dengue fever</td>
<td>120</td>
<td>23</td>
<td>20</td>
<td>79</td>
</tr>
<tr>
<td>Dengue fever can be transmitted by blood transfusion</td>
<td>120</td>
<td>27</td>
<td>23</td>
<td>77</td>
</tr>
<tr>
<td>Dengue fever can be transmitted by sexual intercourse</td>
<td>120</td>
<td>10</td>
<td>9</td>
<td>85</td>
</tr>
<tr>
<td><strong>Prevention</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Window screens and bed nets reduce mosquitoes</td>
<td>120</td>
<td>78</td>
<td>40</td>
<td>66</td>
</tr>
<tr>
<td>Insecticide spray reduces mosquitoes and prevents dengue</td>
<td>120</td>
<td>89</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>Covering water containers reduces mosquitoes</td>
<td>120</td>
<td>115</td>
<td>3</td>
<td>98</td>
</tr>
<tr>
<td>Removal of standing water can prevent breeding of mosquitoes</td>
<td>120</td>
<td>109</td>
<td>8</td>
<td>93</td>
</tr>
<tr>
<td>Mosquito repellents prevent mosquito bite</td>
<td>120</td>
<td>111</td>
<td>8</td>
<td>93</td>
</tr>
<tr>
<td>Cutting down bushes can reduce mosquitoes and dengue</td>
<td>120</td>
<td>105</td>
<td>14</td>
<td>88</td>
</tr>
</tbody>
</table>
Table 3. Dengue prevention practices, Myanmar, 2017

<table>
<thead>
<tr>
<th>Prevention practice</th>
<th>Total</th>
<th>Always</th>
<th>Usually</th>
<th>Sometimes</th>
<th>Never</th>
<th>No response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Spray</td>
<td>120</td>
<td>5</td>
<td>4</td>
<td>10</td>
<td>8</td>
<td>66</td>
</tr>
<tr>
<td>Professional pest control</td>
<td>120</td>
<td>42</td>
<td>35</td>
<td>37</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>Window screen</td>
<td>120</td>
<td>15</td>
<td>13</td>
<td>11</td>
<td>9</td>
<td>22</td>
</tr>
<tr>
<td>Fan</td>
<td>120</td>
<td>13</td>
<td>11</td>
<td>15</td>
<td>13</td>
<td>41</td>
</tr>
<tr>
<td>Bed nets</td>
<td>120</td>
<td>79</td>
<td>66</td>
<td>18</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Remove standing water</td>
<td>120</td>
<td>42</td>
<td>35</td>
<td>36</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>Mosquito coil</td>
<td>120</td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>8</td>
<td>64</td>
</tr>
<tr>
<td>Cover water containers</td>
<td>120</td>
<td>78</td>
<td>65</td>
<td>20</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Cut down bushes around the house</td>
<td>120</td>
<td>37</td>
<td>31</td>
<td>32</td>
<td>27</td>
<td>39</td>
</tr>
<tr>
<td>Temephos</td>
<td>120</td>
<td>44</td>
<td>37</td>
<td>33</td>
<td>28</td>
<td>37</td>
</tr>
</tbody>
</table>

Community acceptance of temephos

Table 4 presents the community acceptance of temephos in 114 individuals (95%) who used it. Of these, instructions to use temephos were received by 113 (99%). Acceptance of smell was 35% and acceptance of turbidity was only 12%. Perception on reduced mosquito density was 68%. The number of respondents who wanted to recommend these measures to their neighbours was low (8%).

Susceptibility of Aedes spp. larvae to temephos

The study assessed the susceptibility of Aedes spp. larvae to temephos in six states/regions – Yangon, Ayeyarwady, Bago, Mon, Kayin and Rakhine. Aedes spp. larvae were collected from 60 breeding sites. Figs. 2 and 3 present the findings on the temephos susceptibility. Mortality was 30–39% with 0.5 and 1 ppm in ½ hour, which reached ~80% in 1 hour and 100% in 2 hours. Yangon region started to show mortality after 30 minutes and 100% mortality occurred within 1 hour. The first ½ hour mortality in Ayeyarwady region had 0% mortality,
30–40% mortality occurred in 1 hour and 100% mortality occurred in 2 hours. Mortality in Bago, Mon, Kayin and Rakhine reached 100% in 2 hours. In 24 hours, 100% mortality occurred in the six states/regions; therefore, temephos is still susceptible to *Aedes* spp. larvae in these states/regions.

**Table 4.** Community acceptance of temephos, Myanmar, 2017

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total</th>
<th>Yes</th>
<th>%</th>
<th>No</th>
<th>%</th>
<th>No response</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvicide used</td>
<td>120</td>
<td>114</td>
<td>95</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Instructions provided</td>
<td>114</td>
<td>113</td>
<td>99</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Perception on reduced mosquito density</td>
<td>111</td>
<td>76</td>
<td>68</td>
<td>9</td>
<td>8</td>
<td>26</td>
<td>23</td>
</tr>
<tr>
<td>Recommendation of larvicide to neighbours</td>
<td>111</td>
<td>9</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>102</td>
<td>92</td>
</tr>
<tr>
<td>Acceptance of smell</td>
<td>111</td>
<td>39</td>
<td>35</td>
<td>20</td>
<td>18</td>
<td>52</td>
<td>47</td>
</tr>
<tr>
<td>Acceptance of turbidity</td>
<td>111</td>
<td>13</td>
<td>12</td>
<td>46</td>
<td>41</td>
<td>52</td>
<td>47</td>
</tr>
</tbody>
</table>

**Fig. 2.** Effectiveness of temephos 1% sand granules with *Aedes* spp. larvae, 2017. Mortality was 30–39% with 0.5 and 1 ppm in ½ hour, which reached ~80% in 1 hour and 100% in 2 hours.
Community acceptance of temephos (1% sand granules formulation) and its susceptibility to Aedes spp. larvae in Myanmar, 2017

Fig. 3. Effectiveness of temephos 1% sand granules with Aedes larvae by states/regions, 2017. Mortality with 0.5 and 1 ppm reached 100% in 1 hour in Yangon, Mon and Kayin; in 2 hours in Ayeyarwady and Rakhine; and it reached 100% in 24 hours in all states/regions.

Discussion

The present study was conducted to understand the community knowledge of cause, transmission and prevention, prevention practices of dengue and acceptance and susceptibility of Aedes larvae to temephos sand granules. The knowledge of important cause, transmission and prevention was over 80% in most of the areas assessed. A similar study done in Viet Nam showed the average knowledge score of 4.6 of 19 assessed (10). A study in Malaysia has shown that the community had high knowledge and the level of knowledge about dengue
is directly proportional to the dengue transmission in the community (11). A study done in Ethiopia has shown that only 31% of the respondents exhibited high knowledge, which was lower than that in Myanmar (12). Respondents in the states/regions studied had knowledge that dengue is transmitted by blood transfusion (22.7%), which has been reported in a few cases (13). Misconceptions about transmission were found around personal contact, and sexual intercourse in minimal respondents. The National Dengue Prevention and Control Programme publishes millions of interpersonal and communication materials targeting the urban and rural communities in the country to raise awareness and help practice protective behaviours. These materials need to focus on the gaps identified in this study, particularly to clear the misconceptions about transmission.

The most common dengue prevention practice was the use of temephos, which matched with the government supplies of temephos to the communities. This shows that the programme was successful in distributing temephos to the communities, provide instructions on its use and its impact on dengue mosquitoes. Communities also practiced spraying (68%) and covering water containers (88%), environmental cleaning (88%), and removing standing water (89%). Studies have shown that cleaning of the environment has a positive impact on lowering dengue cases (14). Less frequently practiced was the use of window screen, which could be related to the economic status of the respondents – this aspect was not considered in this study. The uptake of dengue prevention practices is also linked to the demographic background (including socioeconomic status), perception of susceptibility to dengue, perceived density of mosquitoes in the neighbourhood and knowledge about specific prevention practices (15, 16). Future studies could explore the factors affecting the uptake of various dengue prevention practices.

The overall acceptability of temephos use in the community was high, which is linked to the perception of reducing the density of the dengue vector despite the dislike for smell and turbidity. Similar challenges have been noticed on the use of temephos in communities in other countries (5,6). Advocacy efforts need to be in place to motivate communities to use it.

This study clearly showed that Aedes spp. larvae were 100% susceptible to temephos in the six states/regions studied. Yangon region started to show mortality after 30 minutes and 100% mortality occurred within 1 hour. However, other states such as Ayeyarwady region had 0% mortality in 30 minutes, which reached to 20% to 32% mortality in 1 hour and 100% mortality occurred after 2 hours. Mortality in Bago, Mon, Kayin and Rakhine also did not reach 100% in the first hour as in Yangon. It is important to monitor the susceptibility to temephos since it has been used in large quantities in these states/regions – it is the only larvicide that is being currently used. The study did not look into the cause–effect relationship – the use of temephos and its direct impact on dengue cases and deaths. However, it was observed that dengue cases and deaths declined drastically in 2018 in comparison to 2017 at the states/regional levels by 36% and 62%, respectively (1). It is worthwhile to study the impact of temephos on dengue cases and deaths at the community level and also to associate it with the level of dengue knowledge and practices. We also did not investigate the application
of temephos on the reduction of entomological indices. Studies have shown good results of a single intervention temephos on the reduction of entomological indices over mixed interventions (17). The community effectiveness of temephos also needs to explore factors such as the quality of delivery, water turnover rate, type of water, and environmental factors such as organic debris, temperature and exposure to sunlight, which we did not examine in this study.

In conclusion, the Dengue Prevention and Control Programme conducted mass larviciding with temephos 1% sand granules in six states/regions during January–June 2017. The respondents had high knowledge of cause, transmission and prevention of dengue, and some misconceptions prevailed about transmission. The community also practiced protective behaviours with high acceptability to use temephos despite its dislike for smell and turbidity. Temephos was 100% effective against Aedes spp. larvae. The National Dengue Prevention and Control Programme should continue to monitor its susceptibility and make efforts to raise awareness to manage misconceptions.

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**Role of investigators**

NYYL: Principal Investigator, conception/design of the protocol, acquisition of data, data analysis/interpretation, drafting/critically reviewing the paper, giving approval for the final version to be published.

BT: Conception/design of the protocol, data analysis/interpretation, critically reviewing the paper, giving approval for the final version to be published

AAM: Conception/design of the protocol, critically reviewing the paper

TH: Conception/design of the protocol, data analysis/interpretation, drafting/critically reviewing the paper, giving approval for the final version to be published.

SSW/T/ON/AT/NL/AMMK/MMT/SKK/SYN/TMS/WPT/ZL: Conception/design of the protocol, data analysis/interpretation, critically reviewing the paper, giving approval for the final version to be published.
Disclosures

BT, SSW, SKK, ZL are staff members of the World Health Organization (WHO). The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions, policy or views of WHO. There are no conflicts of interest to report.

References


Preliminary study on underlying biochemical mechanism of insecticide resistance of *Aedes albopictus* in Thiruvarur district, Tamil Nadu, India

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Abstract

Major vector-borne diseases (VBDs) in India transmitted by *Aedes* mosquitoes are dengue, and chikungunya. Until a suitable vaccine is available, vector control is the main option to prevent or reduce transmission of the disease. *Aedes albopictus* and *Aedes aegypti* are known to cause arboviral diseases. In the current study, *Ae. albopictus* mosquitoes from Thiruvarur district of Tamil Nadu, India were investigated for the susceptibility status to various insecticides. Further, an attempt was made to understand the biochemical and resistance mechanisms via *kdr* (knockdown resistance) mutation studies. For this purpose, field-collected immatures of *Ae. albopictus* reared to F1 generation were exposed to DDT, deltamethrin and malathion, at various concentrations by following the WHO protocol. For biochemical assays, monoxygenases, esterases, insensitive acetylcholinesterases (iAChE) and glutathione S-transferase (GST) enzyme assays were performed; this was followed by an allele-specific-PCR (AS-PCR) assay for detection of *kdr* mutation to check F1534C mutation. Our results showed that out of the three insecticides tested, *Ae. albopictus* were resistant to DDT but fully susceptible to malathion and deltamethrin. Biochemical assays indicated slight elevation in the GST level. Monoxygenase, acetylcholinesterase and esterase activities were found not to have been involved in conferring DDT resistance in the field strain of *Ae. albopictus*. F1534C mutation at segment 6 of domain III of *kdr* by AS-PCR in *Ae. albopictus* showed the absence of mutation. Further sequencing of samples did not show mutation in the voltage-gated sodium channels (vGSC) gene. To date, no information is available on the susceptibility status of *Ae. albopictus* from Thiruvarur district. The susceptibility status and information on underlying mechanisms presented here help in developing suitable insecticide-based vector control strategies for disease management.

Keywords: *Ae. albopictus*; susceptibility status; DDT; malathion; deltamethrin; *kdr* mutations; biochemical enzymes.

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Introduction

Aedes-borne diseases such as dengue, chikungunya, yellow fever and Zika are rapidly spreading vector-borne diseases (VBDs) worldwide (1). *Aedes albopictus* (Skuse, 1894) and *Aedes aegypti* (Linnaeus, 1762) are the vectors for arboviral diseases. Historically, *Ae. albopictus* occurred in tree holes, referred to as “forest mosquito”, which spread throughout the Oriental region from the tropical and subtropical regions of South-East Asia, Pacific and Indian Ocean Islands and other countries (2). Vector control is the only way to reduce the transmission of various diseases until a suitable vaccine is available for such arboviral diseases (1). Efforts for planned urbanization control of larval breeding and educational awareness have been made to control the vector populations. The application of chemical insecticides is one effective way in the vector control programme (3). Four classes of insecticides are widely used in vector control programmes – organochlorines, organophosphates, carbamates and pyrethroids (4). Regular use of insecticides has led to the development of resistance against the insecticides, which reduces the efficacy of vector control programmes (5). Several studies have summarized the insecticide resistance status in *Ae. albopictus* worldwide such as Thailand (6), Papua New Guinea (7), Malaysia (8), China (9) and India (10–13). The development of resistance to insecticide and the underlying mechanisms among mosquitoes have been well documented, which are of four different categories. Metabolic resistance is due to the increased detoxification of enzymes such as P450-monoxygenases, glutathione S-transferases (GST), and carboxylesterases (14,15). Target-site resistance is due to the changes in binding sites of insecticide that makes the target site incompatible for binding. An example of such changes is mutations in the voltage-sensitive sodium channel (*vssc*) gene (16). Cuticular resistance occurs because of reduced penetration of insecticides due to modifications in the insect cuticle or digestive tract linings (15). Behavioural resistance is primarily the avoidance of insecticide contact by the vector mosquitoes. Metabolic-based resistance confers the mutation either in the enzyme protein sequence or non-coding regulatory region. It involves three groups of detoxifying enzymes, viz. monooxygenase, GST and esterases (ESTs) (17). A point mutation in voltage-gated sodium channel (*vgsc*) is the most common cause of target site resistance, which is also known as “knockdown resistance” (*kdr*). Various point mutations in the *vgsc* gene (*I1011V, I1011M, V1016I and V1016G*) have been reported in pyrethroid and DDT resistance in *Ae. aegypti* (16,18,19). In Thailand and Viet Nam, F1534C mutation in the *vgsc* gene has been found in *Ae. aegypti* (20). The same mutation of F1534C was reported from Singapore in *Ae. albopictus* (21) and from Odisha state in India (22). Organophosphate insecticide resistance was reported to be associated with single nucleotide polymorphisms in the acetylcholinesterase-1 (ace-1) gene in the encoded acetylcholinesterase enzyme (23).

In view of the above information, a study was conducted to assess the status of insecticide susceptibility and underlying mechanisms responsible for conferring resistance to different classes of insecticides in *Ae. albopictus* collected from Thiruvarur district of Tamil Nadu.
Material and methods

Study site

Thiruvarur district is situated on the southern Indian coastal region of the river Kavery (3 metres above sea level) of Tamil Nadu state. The larval collection for the study was done in all the ten blocks of the Thiruvarur districts, namely Koradacheri, Kottur, Kudavasal, Mannargudi, Muthupet, Nannilam, Needamangalam, Thiruthiraipoondi, Thiruvarur and Valangaiman. The study was carried out in the Vector Biology Research Laboratory (VBRL), Department of Life Sciences at Central University of Tamil Nadu (CUTN) and National Institute of Malaria Research (NIMR), New Delhi. The district is mostly rural where people live in small houses and follow water-storage practices.

Mosquito collection and rearing: Collected immature (larvae and pupae) from the field were brought to VBRL at CUTN for rearing. Emerged mosquitoes were identified morphologically to species. Adults of F1 generation were used for the insecticide susceptibility test. These were also subjected to biochemical assays for GST, esterases ($\alpha$ and $\beta$), mixed function oxidases (MFOs), insensitive acetylcholinesterase enzymes and protein estimation to determine the resistance mechanism.

Due to the unavailability of a susceptible Ae. albopictus strain, Anopheles culicifacies was used as the proxy susceptible strain in this study. This strain was collected from Dehra village in Uttar Pradesh in the year 1990 and is being colonized in the insectary at NIMR, Delhi without insecticide selection pressure. This strain was tested periodically for susceptibility by exposing it to the prescribed discriminatory dosage of insecticide impregnated papers (DDT 4%, malathion 5% and deltamethrin 0.05%) using the WHO method and kit.

Adult bioassay

Insecticide susceptibility assays were performed according to the WHO recommended method (WHO, 1998) and using a WHO susceptibility kit. A total of 100 mosquitoes in four test replicates were exposed to DDT 4% (organochlorine), malathion 5% (organophosphate) and deltamethrin 0.05% (synthetic pyrethroid) along with two control replicates of total 50 mosquitoes.

Biochemical assays

Three-to-five days old sugar-fed female adult mosquitoes of F1 generation were used for the biochemical assays. Monoxygenases, ESTs and insensitive acetylcholinesterases (iAChE) enzyme assay and GST enzyme assay were performed following WHO guidelines (5).
**Protein assay**

Protein was measured in an individual mosquito with 10 µl of insect homogenate by using the standard Bradford method. Absorbance was measured at 570 nm after incubation of 5 minutes.

**Allele-specific PCR assay for detection of kdr mutation**

DNA of adult *Ae. albopictus* was extracted from Thiruvarur district. Insecticide was exposed (DDT 4%) to mosquitoes (both dead and live). AS-PCR was carried out to know the *kdr* allele for F1534C mutation at segment 6 of domain III following Yanola *et al.* (2011) (20).

**Genotyping of voltage-gated sodium channel (vgsc) gene**

Sequencing was performed for further validation of AS-PCR results in both dead and live mosquitoes for verification of *kdr* allele for F1534C mutation. The sequence of segment 6 from domain III of the vgsc gene containing the codon for F1534C mutation was amplified by PCR using primers followed by Kasai *et al.* (2011) (21).

**Statistical analysis**

Adult mortality was corrected by using Abbott’s formula. According to the 1998 WHO criteria, mortality percentage was determined against different classes of insecticides (24). Knockdown time (KdT50 and KdT95) values were calculated by log-probit linear regression (25).

**Results**

**Insecticide susceptibility assays**

The susceptibility status of *Ae. albopictus* against DDT 4%, malathion 5% and deltamethrin 0.05% is shown in Table 1. The data indicate that *Ae. albopictus* was resistant to DDT (4%) in Thiruvarur district. The per cent mortality was 41% to DDT and was 100% susceptible to malathion and deltamethrin.
Table 1. Insecticide susceptibility status

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>No. of mosquitoes exposed</th>
<th>Mortality (%)</th>
<th>KdT50 (min)</th>
<th>KdT95 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT (4%)</td>
<td>150</td>
<td>41</td>
<td>69.5 (58.8–101.5)</td>
<td>109.9 (86.0–190.8)</td>
</tr>
<tr>
<td>Malathion (5%)</td>
<td>150</td>
<td>100</td>
<td>28.3 (26.0–30.2)</td>
<td>46.2 (42.9–50.9)</td>
</tr>
<tr>
<td>Deltamethrin  (0.05%)</td>
<td>150</td>
<td>100</td>
<td>22.9 (18.4–26.0)</td>
<td>36.1 (31.2–49.2)</td>
</tr>
</tbody>
</table>

**Biochemical assays**

**Acetylcholinesterase assay:** In AchE assays, the population with more than 30% AchE activity are considered sensitive, i.e. they do not have iAchE, which accounts for 69% of the population, while the remaining 31% will be considered as the population with insensitive AchE (Fig. 1).

**Fig. 1.** Acetylcholinesterase enzyme activity levels of *Ae. albopictus* as measured by absorbance for field strain and laboratory strain

**Esterase assay:** In alpha-esterase enzyme assay, 94% of the field population showed activity within the susceptibility threshold value as determined for susceptible laboratory strain and the remaining 6% showed activity beyond the susceptibility threshold value, indicating minimum involvement of this enzyme activity in conferring resistance (Fig. 2).
In beta-esterase enzyme assay (Fig. 3), 89% of the field population showed activity within the susceptibility threshold value as determined for the susceptible laboratory strain and the remaining 11% showed activity beyond the susceptibility threshold value, indicating minimum involvement of this enzyme activity in conferring resistance.
**Monooxygenase assays:** In monooxygenase assay, enzyme assay, 70% of the field population showed activity within the susceptibility threshold value as determined for susceptible laboratory strain and the remaining 29% showed activity beyond the susceptibility threshold value, indicating minimum involvement of this enzyme activity in conferring resistance (Fig. 4).

*Fig. 4.* Monooxygenase enzyme activity levels of *Ae. albopictus* as measured by absorbance for field strain and laboratory strain

**Glutathione S-transferase assay:** In GST assays, results indicate that there is no involvement of elevated GST activity in conferring DDT resistance in the field-collected strain as both the populations have shown a similar activity profile (Fig. 5).

*Fig. 5.* GSTs detoxification enzyme activity levels of *Ae. albopictus* as measured by absorbance for field strain and laboratory strain
Results of genotyping for F1534C kdr mutation by AS-PCR in Ae. albopictus showed the absence of this mutation. Further validation of mutation sequencing was done commercially, which did not reveal any variation in the earlier reported mutation in the vgsc gene.

**Discussion**

The insecticide resistance status of Ae. albopictus has been studied in different places in India and worldwide. The studies have interpreted that continued usage of insecticides resulted in the development of resistance in mosquito vectors in many countries and the information on mosquito vector insecticide susceptibility status and underlying resistance mechanisms is important to suggest appropriate insecticide-based vector control strategies. This study provides such information on insecticide susceptibility status in Ae. albopictus population in Thiruvarur district of Tamil Nadu. According to the WHO recommendation, mortality >98% is considered as susceptible, 91–97% as possible resistance and <90% as confirmed resistant after 1 hour exposure to insecticide and 24-hour holding post-exposure. In our study, the susceptibility status of Ae. albopictus showed 100% mortality against deltamethrin and malathion, while mortality against DDT was 41%, clearly indicating the resistance.

To date, there has been no information on the susceptibility status of Ae. albopictus from Thiruvarur district. Nevertheless, some previous studies have also recorded resistance to DDT for Ae. albopictus in India and other parts of the world (6,22,26). This study also showed the susceptibility of Ae. albopictus to organophosphate (malathion) and pyrethroid (deltamethrin), which indicates that these insecticides can be used in public health programmes for vector control in management of arboviral diseases.

Biochemical assays for adults indicated a slight elevation in the GST level. The elevated GST level corresponds to resistance towards DDT (27). Previous studies have shown elevated levels of GST and a role in conferring DDT resistance (14,28). In this study, both the susceptible and resistant Ae. albopictus populations showed a similar profile of activity with slight enhancement in the GST level in the resistant strain, but with no substantial increment in activity to indicate involvement in conferring resistance. Hence, some other substrates such as DCNB (2, 5-Dichloronitrobenzene) and iodoethane can be used to determine the possible role of GST in conferring resistance against DDT (29). Other enzymes such as monooxygenase, acetylcholinesterase and esterase activities were found not to have been involved in conferring DDT resistance in a field strain of Ae. albopictus.

In this study, the F1534C mutation at segment 6 of domain III, kdr mutation studies by AS-PCR in Ae. albopictus in the population of Thiruvarur district showed the absence of mutation. Further sequencing of samples did not show a mutation in the vgsc gene. Similar results of F1534C mutation were reported from various urban locations of India (30). Surprisingly, kdr mutation with high frequency has been reported in Singapore (21) and India (22). In Ae. aegypti, kdr mutation is one of the major mutations reported in populations from different parts of India (31).
Conclusion

Our study examined insecticide susceptibility of \textit{Ae. albopictus} in Thiruvarur district. The species is reported resistant to DDT and susceptible to malathion and deltamethrin. Susceptibility status and information on underlying mechanisms will help in developing suitable insecticide-based vector control strategies for disease management.

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Preliminary study on underlying biochemical mechanism of insecticide resistance of Aedes albopictus in Thiruvarur district, Tamil Nadu, India


Dengue and Chikungunya Outbreak Containment: A Success Story of Ranchi in Jharkhand, India

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Abstract

Jharkhand, a state of India reported dengue cases in 2006 followed by an outbreak of dengue in 2010 from Jamshedpur town of East Singhbhum district. During 2011, the district reported chikungunya case for the first time which was followed by reports of dengue and chikungunya cases from other parts of the state. However, in subsequent years, both dengue and chikungunya cases increased and spread to newer localities in other districts. During 2018, the state experienced outbreak mainly from two districts i.e., East Singhbhum and Ranchi (the state capital). The identification of hotspot and timely action through supervised implementation of ‘OCTALOGUE’ strategy as per National guidelines, India resulted in containing the outbreak successfully within a period of one month. The success story of outbreak containment through intensified surveillance, case management, vector management, outbreak response, capacity building, IEC/BCC, inter-sectoral collaboration and monitoring has been discussed in the paper.

Keywords: Dengue, Chikungunya, Outbreak containment.

Introduction

Dengue and Chikungunya are outbreak prone arboviral diseases which have spread throughout India. In some parts of country, these are seasonal and cyclical contributing to annual outbreaks¹⁻³. Jharkhand state has been reporting dengue cases since 2006⁴. However, in 2010 two dengue sero-positive cases were reported from Jamshedpur town of district East Singhbhum in the state. Following this, three Sentinel sites viz. Rajendra Institute of Medical Sciences (RIMS), Ranchi; Mahatma Gandhi Memorial (MGM) College and Hospital, Jamshedpur and Patliputra Medical College and Hospital (PMCH), Dhanbad were identified in the state for diagnosis and treatment of dengue and chikungunya during 2011⁵. During 2013,
outbreak of chikungunya occurred in the state for the first time with reporting of 399 positive cases (331 from Jamshedpur and 68 from Ranchi). Thereafter, sporadic cases were reported from different districts of the State. In 2015, two more sentinel sites one each in West Singhbhum and Simdega districts were identified. Jamshedpur town remained focus but sporadic cases were also reported from other parts of the State. During 2018, the State had reported an outbreak of both the diseases with 463 dengue and 851 chikungunya cases. Ranchi being state capital and nearest to Jamshedpur town also experienced sudden upsurge in dengue and chikungunya cases in 2018 and reported 177 dengue and 756 chikungunya cases. Jamshedpur town of E. Singhbhum district reported 67 dengue with no chikungunya cases and other nearby district Hazaribagh reported 18 dengue and 47 chikungunya cases. The known vector of dengue and chikungunya i.e. *Aedes aegypti* was prevalent throughout the city along with *Ae. Albopictus*. For containment of sudden upsurge, all eight elements of National strategy for prevention and control of Dengue and Chikungunya called as ‘OCTALOGUE’ were implemented in the affected Hindpiri area of the city as per Mid Term Plan. Accelerated efforts with deployment of committed human resource has resulted in reducing the container index for *Aedes* breeding in affected areas from 32% on 6th August 2018 to 5% on 31st August 2018. Following which the cases of dengue and chikungunya reduced significantly. The analysis of the intensive containment operation and importance of source reduction in containing outbreak of dengue and chikungunya has been discussed in this paper.

**Materials and methods**

The outbreak area Hindpiri, which is in the mid of Ranchi city and lies at 23.3441*N & 85.3096*E was demarcated, It is densely populated with 1.15 lakh population, and covers an area of about 5 to 8 km of diameter (Fig-1). All the eight elements of ‘OCTALOGUE’ of National strategy for prevention and control of dengue and chikungunya viz. surveillance, case management, vector management, outbreak response, capacity building, Information, Education & Communication (IEC)/ Behaviour Change Communication (BCC), inter-sectoral collaboration and monitoring, supervision and reporting were implemented to contain the outbreak.

The first element of the strategy i.e. Surveillance included door to door visit in the affected areas through 5 teams each having 10 members. The teams carried out fever surveillance and entomological surveillance simultaneously.

Case management was initiated by setting 4 Medical camps with a team of 3 doctors and 3 paramedical staffs in each camp. These camps were functioning for a month in and around affected areas. Ambulances were provided in the camps for facilitating immediate referral followed by case management. Laboratory technician was provided in each camp for collection of blood sample of all suspected cases. Collected blood samples were sent to
identified Sentinel site at RIMS, Ranchi for serological confirmation through IgM ELISA Test. Line-list of all cases (both suspected & confirmed) was maintained and all dengue positive patients were followed up for complications for two weeks.

Vector management included source reduction and anti-larval work with intensive inter personal communication (IPC). Community mobilization was also done during surveillance. All water storing containers for household use including clay, ceramic and cement water jars, metal drums/barrels, and smaller containers storing fresh water or rainwater and discarded containers of plastic, ceramic and metals were checked. Uncovered water containers were identified as major potential breeding sources in the area. All water containers were emptied, scrubbed in front of surveillance team and water was replenished in containers through Municipal water tankers. Municipality vehicle was arranged to move with the team to remove all discarded utensils/containers thrown at the backyard of houses acting as potential breeding sources. Besides, strict directives issued to households to cover all water containers, the teams were instructed to ensure the implementation of these directives. The breeding sites/containers were identified, enlisted. Unused containers were scrubbed and kept upside down. A container containing water was considered as wet container and any wet container containing immature (larvae, pupae or both) stages of Aedes mosquito was
considered as positive container. All kinds of indoor and outdoor breeding habitats were examined to collect the *Aedes* immature stages using dippers. The collected immature stages were counted. Penalty of INR 100 to INR 1000 was imposed by Enforcement Officer of Municipal Corporation, Ranchi in the houses found positive in subsequent round of surveillance. Larviciding was done with Temephos and Diflubenzuron whereas anti adult measures were undertaken by instituting thermal fogging in coordination with Municipal Corporation using deltamethrin (kingfog) available with local authority but later cyphenothrin 5% was used by vehicle mounted fogging machine. Community was motivated for personal protection measures like wearing full sleeves cloths, using mosquito nets during day time also and use of repellents to protect themselves from mosquito bites. Long Lasting Insecticidal Nets (LLINs) were distributed to positive patients to prevent transmission to others and any re-infection of the disease.

Outbreak response was immediately put in action by forming Emergency Action Committee (EAC) under the Chairmanship of Deputy Commissioner including representatives of Health Department, Municipal Corporation and District Education Officer as members. The health team supported by Municipal team moved in the affected areas for joint interventions. Rapid Response Team (RRT) at State and District level were formed for situation analysis on daily basis and taking immediate measures. The district preparedness in terms of human resource including Medical Officer, paramedical, surveillance worker and vector control persons with logistics were ensured. Management of print and electronic media was done by briefing them daily on activities carried out for control of dengue and chikungunya to develop public trust. Capacity building of Ranchi Municipal staff was done for vector surveillance, identification of mosquito larvae and reporting. The training also included practical demonstration in the field during door to door surveillance for identification of mosquito larva, source reduction/elimination and reporting. Capsule training of Medical Officers on case management was also arranged immediately at State HQ by involving master trainers from medical college, trained at National level.

IEC/BCC/Inter-personal communication (IPC) was taken up in massive way by conducting School awareness Programme in 124 schools of Ranchi ensuring covering of all 36 schools in affected area. Miking and announcements were done through religious leaders for prevention of dengue and chikungunya in addition to IPC and Handbill distribution during door to door surveillance. Road march (rallies) by officers, prominent persons and local leaders were undertaken repeatedly for making the campaign as movement. In addition to advertisement through newspaper and TV, a mobile vehicle as IEC Van was exclusively deputed to move in and around the affected area on daily basis to announce the risk and preventive measures in local language.

Efforts were made to strengthen Inter-sectoral coordination to generate awareness and to empower different sectors to share responsibilities. The State Task Force on vector borne diseases including representatives of 9 different departments viz., Urban Development,
Education, Road Construction, Panchayati Raj, Drinking Water & Sanitation, Agriculture, Animal Husbandry & Cooperative, Rural Development, Information & Public Relation and Women, Child Development & Social Welfare had an emergency meeting to seek cooperation from all stakeholders for control of outbreak. Advocacy of local leaders and religious leaders was done for their cooperation during surveillance activities.

Monitoring, supervision and reporting were undertaken meticulously. The commitment of all officials was ensured by daily briefing and moral boosting. Daily reporting of clinical examination in medical camps, blood samples collected, numbers positive cases of dengue and chikungunya and entomological surveillance data were ensured. Supervisory teams from state and district were deputed in field for situation analysis on daily basis and verification of collected reports. Every evening, data generated on daily basis like reporting of cases, larval indices and logistics status was monitored at district and state level to initiate additional measures as per need in addition to routine activities.

Results and discussion

The containment measures were initiated in Ranchi town after reporting of single case each of dengue and chikungunya on 26.7.2018. Daily reports of cases and Aedes breeding from first August 2018 till 31.8.2018 were analysed. However, the intensified implementation and monitoring continued in affected area and its surrounding buffer zone till first week of September when the cases declined. During the month of August, Ranchi district as a whole reported a total of 87 dengue cases and 551 chikungunya cases. Out of those, the Hindpiri area reported 58 dengue cases without any mortality and 489 chikungunya cases. The chikungunya cases started rising from 1st August and reached to peak on 8th August and thereafter showed declining trend with fluctuations. Dengue cases though remained at low level also showed maximum cases (16) on 8th August coinciding the peak of chikungunya. The seasonal peak of these arboviral diseases has also been well established. The containment measures could slow down the transmission. Cases of both the diseases started declining and reached at low level by the end of August 2018 (Fig 2). Though intensive campaign was stopped, follow-up was continued in subsequent months. The co-infection of both dengue and chikungunya was observed in 44 cases (4.1%). Co-infection to the extent of 9.54% has been reported from Punjab, India.

Entomological surveillance was undertaken simultaneously and the data is indicated in Table 1. Daily checking of breeding could not be done due to other operational issues. The larval survey was done for 14 days during the period of 6-31 August, 2018. A total of 1614 houses out of 6256 were found positive for Aedes breeding. However, 21097 wet containers were checked and 2372 were found positive for the Aedes breeding. The House index was reduced to 17 from 49, whereas, the Container Index was reduced to 7 from 32 till containment of the outbreak. Breteau Index was initially 186 on 26 July and 69 on
Fig. 2. Epidemiological trend of Dengue and Chikungunya cases in the outbreak area

Table 1. Larval Indices in Hindpiri Area of Ranchi Town

<table>
<thead>
<tr>
<th>Date</th>
<th>House Index</th>
<th>Container Index</th>
<th>Breteau Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>06-08-2018</td>
<td>49</td>
<td>32</td>
<td>69</td>
</tr>
<tr>
<td>07-08-2018</td>
<td>32</td>
<td>10</td>
<td>29</td>
</tr>
<tr>
<td>08-08-2018</td>
<td>27</td>
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<tr>
<td>09-08-2018</td>
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<td>11-08-2018</td>
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<td>12-08-2018</td>
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<td>13-08-2018</td>
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<td>16-08-2018</td>
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<tr>
<td>17-08-2018</td>
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<td>18-08-2018</td>
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<td>34</td>
<td>22</td>
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<td>30-08-2018</td>
<td>17</td>
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<td>33</td>
</tr>
<tr>
<td>31-08-2018</td>
<td>8</td>
<td>5</td>
<td>15</td>
</tr>
</tbody>
</table>
6 August which was considered to be at high risk for transmission. However, by the end of the month, it was reduced to 15. The potential breeding sources for *Ae. aegypti* as stated earlier were containers storing water for household use including clay, ceramic & cement water jars, metal drums/barrels, and smaller containers storing fresh water or rainwater 8-13.

Media Management was done by announcing the epidemic early, communicating with the public, providing accurate and specific information about what people can do to make themselves and their community safer. This gave people a sense of confidence and responsibility for their health and safety, which in turn prepared them to react to the risk with more reasoned responses. During School awareness programme in 124 schools of Ranchi, all the 36 schools in affected area (Hindpiri) were covered which also helped in generating awareness at faster speed among community.

**Conclusion**

Dengue and Chikungunya are two paradigmatic examples of emerging infections, showing similar epidemic dynamics. Because of increasing population movement and wide geographical distribution of vector mosquito, the endemic areas for both these diseases are expanded14–17 that was previously considered disease-free. The observations made during the outbreak containment explained in the paper also corroborates as Jharkhand state had not reported dengue cases before 2006 when 13 cases were reported4. The spread has been reported to neighboring district possibly due to movement of people and also availability of potential breeding sites for both the *Aedes* vectors. However timely intervention taking multiple approaches in terms of vector control17, disease management and intensive community mobilization may contain any fulminating outbreak and can prevent associated morbidities and mortalities. The timely actions and effective inter departmental coordination discussed in this communication played major roles in containment of outbreak of dengue and chikungunya in the study area. Owing to the threat posed by such infections, it is essential to explore the feasibility of innovative mosquito control strategies and its adoption to control/contain outbreak in critical and resource constraint geographical areas.

**Acknowledgements**

The support provided by Municipal Corporation of Ranchi, District officials and their staff during outbreak containment process is highly acknowledged. The role of different departments including media had been very crucial in building the cooperation from students and community which is greatly acknowledged. Authors are thankful to Mr. Rahul Kumar, Data management group of State NVBDCP, Jharkhand for daily reporting, compilation and analysis of data.
References


Epidemiological stratification of dengue in India and strategic challenges
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Abstract
Dengue, with rapidly evolving epidemiology, has become a major public health concern in many parts of the globe including India. Since its emergence in the country, due to congenial underlying factors for geographical spread, all states and Union Territories (UTs) are affected. While observing the trend of dengue disease in India during the past two decades (2000–2019), all states and UTs are stratified into three categories, viz. high-, moderate- and low-burden states. There is a different pattern of disease transmission, occurrence of outbreaks in these three categories of the states based on their frequency, duration, repetition and effect. The number of dengue cases reported by each category and level of urbanization has shown a positive correlation, whereas a negative association has been observed with regard to the increase in the number of diagnostic facilities. The proposed stratification may be helpful in deciding preventive strategies for each category. Although acceleration of efforts and a focused approach is needed for high-burden states, little slackness in low- and moderate-burden states may lead to outbreak/s due to susceptible community, prevalence of vector and large-scale population movement.

Keywords: Dengue; epidemiology; stratification category; burden.

Introduction
Dengue viruses (DENV) are fastest-growing arboviral pathogens of humans causing an estimated 390 million infections worldwide annually (1). These are positive stranded encapsulated RNA viruses belonging to Flaviviridae family (2,3). With rapidly evolving epidemiology, dengue has become a major public health concern throughout tropical and subtropical regions with a 30-fold increase in global incidence during the past five decades (4). Almost half the world’s population lives in countries where dengue is endemic. According to the World Health Organization (WHO), about 50–100 million new dengue infections are estimated to occur annually in more than 129 endemic countries with an increase in the number of countries (5–7). Dengue has been identified as one of the 17 neglected tropical diseases (NTDs) by WHO in its first report on NTDs (2010) (6). Although the full global burden

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of the disease is still uncertain, the patterns are alarming for both human health and the economy (8–11). Every year hundreds of thousands of severe cases arise including 20,000 deaths; 264 disability-adjusted life years (DALYs) per million populations are lost. Dengue is also listed among the 40 emerging diseases of global importance (12,13).

More than 70% (about 1.8 billion) of the population at risk for dengue worldwide lives in Member States of the WHO South-East Asia Region (SEAR) and the Western Pacific Region, which bears nearly 75% of the global disease burden due to dengue (14–17). In SEAR, except the Democratic Peoples’ Republic of Korea, all 10 countries including India are endemic for dengue. The contribution of India to total dengue cases in SEAR has increased from 6% in 2009 to 39% in 2017. Based on the transmission potential for dengue, WHO has categorized the countries in SEAR into three categories – A, B and C. Till 2009, India was under category B in which cyclical epidemics are more frequent, circulation of multiple virus serotypes and geographical expansion is occurring (14–16). WHO grouped India in category “A” countries in 2010 (7) where dengue has become a major public health concern, a leading cause of hospitalization and death among children, hyper-endemicity in urban centres, spreading to rural areas and with multiple virus serotypes in circulation (18,19).

In India, the occurrence of dengue fever was first reported during 1956 from Vellore district in Tamil Nadu (20–21). The first dengue haemorrhagic fever (DHF) outbreak occurred in Calcutta (present Kolkata), West Bengal in 1963 (22–23). Since then, all states/UTs in the country have reported cases and deaths due to dengue (24–27). Repeated outbreaks of dengue have also been reported from many parts of the country (28–42). In the past few years, dengue is increasing and reported from newer areas; this spread is attributed to many underlying factors resulting in expanding its mosquito vector (Aedes spp.) as well as geographical distribution of the virus.

This paper describes dengue as a disease, stratification of states in India based on disease burden, the changing epidemiology over the years and challenges in prevention and control. In view of limited resources for dengue control, this stratification intends to prioritize the high-risk areas for judicious use of available resources and tools.

**Methodology**

**Data collection:** In India, the National Vector Borne Disease Control Programme under the Ministry of Health and Family Welfare, Government of India deals with dengue along with other five vector-borne diseases, viz. malaria, kala-azar, lymphatic filariasis, chikungunya and Japanese encephalitis. At the national level, reports of dengue-positive cases and deaths submitted by the states are compiled and analysed for monitoring the disease trend to identify the outbreak including causes and to take corresponding preventive and responsive measures concerning planning, budget allocation and policy-making (43–44).
Effective prevention and control of dengue requires an active laboratory-based disease surveillance programme to provide early warning for transmission. To augment surveillance and diagnostic facility, a network of 680 Sentinel Surveillance Hospitals (SSHs) with laboratory facilities has been established by 2019 (45). The ELISA-based NS1 and IgM kits are used for confirmation of dengue cases as per the national guidelines (24,43,44,46). These institutes submit their reports for dengue cases including detailed information of patients in a line-list form. Also, some major private hospitals having the ELISA facility for dengue diagnosis submit their reports to the concerned state, which they include in their reports for further submission at the national level.

Data analysis: The data were entered, processed and analysed by using MS Excel 2010. Descriptive analysis was done for various factors such as range, mean, standard deviation (SD), etc. ANOVA test was used for validation of significant difference between dengue cases and deaths in all the three categories. For population, the data available on the Niti Aayog website are used for 2001 and 2011 census (47).

On the basis of the number of cases reported in the past two decades (2000–2009 and 2010–2019), the states were stratified into three categories: high-, moderate- and low-burden states. The data available on various websites related to geographical and population aspects were downloaded and interpreted (47–49). Correlation between various factors and number of reported cases was analysed and the results are discussed.

Results and discussion

Incidence of dengue has progressively increased in India in the past 20 years with geographical spread to newer areas. Recurring outbreaks of varying magnitude have been reported from many states/UTs. There is an 11-fold increase in the number of cases between the first decade (2000–2009) and the next decade (2010–2019) with highest numbers of reported cases (188 401) reported in 2017. The dengue morbidity trend in India from 2000 to 2019 is depicted in Fig. 1.

Transmission of dengue is predominantly influenced by vector density, which is compounded by community water-storage behaviour and environment particularly temperature and rainfall. In India, transmission dynamics of dengue is correlated with monsoon and post-monsoon when preponderance of vector is higher due to abundance of breeding habitats in rainfed containers. The disease mostly exhibits a seasonal pattern at the country level. Cases increase with the onset of monsoon, the increase continues throughout the wet months and reaches its peak during the post-monsoon season and then declines. This pattern is not uniform at the state level due to diverse eco-epidemiological variants within the states (50), which is evident from the perennial transmission in southern and western states.
Based on the transmission dynamics and epidemiological characteristics, the states and UTs (Ladakh bifurcated from Jammu and Kashmir in October 2019 (51), hence not considered as separate in this paper) can be divided into three broad categories in India (Fig. 2); viz. high burden, moderate burden and low burden as follows:

Epidemiological features of these three categories are given below:

(A) High-burden states – Out of 36, a total 15 states (13 states and 2 UTs), viz. Andhra Pradesh, Delhi, Gujarat, Haryana, Karnataka, Kerala, Maharashtra, Odisha, Puducherry, Punjab, Rajasthan, Tamil Nadu, Telangana, Uttar Pradesh and West Bengal are under this category. These contributed maximum dengue cases in the country during the study period and are highly vulnerable. All four serotypes are in circulation in the states under this category.

(1) Dengue endemicity: These states are featured with hyper-endemicity with a cyclical inter-annual pattern of epidemic.

(2) Cases: These 15 states contributed 83.4% to country’s total dengue cases during the past two decades.

(3) Deaths: These high-burden states contributed 82.3% to country’s total dengue deaths during the past two decades.
Epidemiological stratification of dengue in India and strategic challenges

Fig. 2. Map of India showing stratification of states based on dengue burden (Source: NVBDCP)

Table 1. Stratification of states and epidemiological characteristics

<table>
<thead>
<tr>
<th>Category</th>
<th>Disease endemicity</th>
<th>Transmission</th>
<th>Outbreak</th>
<th>Areas/districts affected</th>
<th>Deaths due to dengue</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>High burden</td>
<td>Hyper-endemic</td>
<td>Mostly perennial</td>
<td>Repeated</td>
<td>Majority of districts</td>
<td>Higher</td>
<td>All four</td>
</tr>
<tr>
<td>Moderate burden</td>
<td>Meso-endemic</td>
<td>Seasonal with long transmission window</td>
<td>Occasional</td>
<td>Only few districts affected</td>
<td>Few numbers</td>
<td>Mostly single or two</td>
</tr>
<tr>
<td>Low burden</td>
<td>Hypo-endemic</td>
<td>Seasonal with short transmission window</td>
<td>Rare</td>
<td>Only few districts affected</td>
<td>Nil or only in outbreak year</td>
<td>Single</td>
</tr>
</tbody>
</table>
(4) **Outbreak:** The frequency of outbreaks in high-burden states is more, and states under this category report upsurge/outbreaks every year with high number of cases and deaths due to dengue.

(5) **Population and urbanization:** The 15 high-burden states have 56.43% of total districts of the country and 74.06% of the country’s population resides in these states. During 2001, these states had a total of 74.41% towns of the country, which became 71.17% in 2011.

(B) **Moderate-burden states** – A total of nine states (seven states and two UTs), viz. Assam, Bihar, Chandigarh, Chhattisgarh, Dadra and Nagar Haveli, Goa, Jammu and Kashmir, Madhya Pradesh and Uttarakhand are under this category. These states show seasonal transmission with occasional outbreaks. Only few districts of the states are affected and deaths were also reported due to dengue during the study period. These states have mostly one or two serotypes in circulation.

(1) **Dengue endemicity:** These states are meso-endemic with seasonality having peaks usually during late monsoon or post-monsoon.

(2) **Cases:** Moderate-burden states contributed 0.03–15.0% of country’s total dengue cases during the past two decades.

(3) **Deaths:** States under this category contributed 0–17.7% of country’s total dengue deaths during the past two decades and mostly during the outbreak year.

(4) **Outbreaks:** In these states, few districts reported outbreaks with high number of dengue cases and few deaths, which usually does not repeat although cases may be reported.

(5) **Population and urbanization:** The moderate-burden states have 26.39% of total districts of the country and 20.57% of the country’s population resides in these states. During 2001, these states had a total of 20.53% towns of the country, which became 23.19% in 2011.

(C) **Low-burden states** – This category includes a total of 12 states (nine states and three UTs), viz. Andaman and Nicobar Islands, Arunachal Pradesh, Daman and Diu, Himachal Pradesh, Jharkhand, Lakshadweep, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura. Only few districts of these states are affected. Outbreaks are rare and deaths, if occurred, were in the outbreak year only. These states have only one serotype in circulation.

(1) **Dengue endemicity:** These are hypo-endemic states with a seasonal and short transmission window.
(2) **Cases:** States under this category contributed 0–4.8% of the country’s total dengue cases during the past two decades.

(3) **Deaths:** These states contributed 0–4.7% of the country’s total deaths due to dengue during the past two decades.

(4) **Outbreaks:** The low-burden states report only few outbreaks with sudden increase in number of cases and deaths due to dengue in one or two districts mostly in outbreak year only.

(5) **Population and urbanization:** These states have 17.19% of total number of districts of the country and 4.52% of the country’s population resides in these states. During 2001, these states had a total of 5.05% towns of the country which increased to 5.64% in 2011.

The year-wise contribution of dengue cases in the country’s total by all these three categories during 2000–2019 is shown in Fig. 3 and deaths are shown in Fig. 4. On analysis, it has been observed that as per the Census of 2001, the population density in high-burden states is in the range 165–9340 persons per sq.km, which increased to 201–11 297 during 2011. Similarly, in moderate-burden states the range was 100–7900 persons per sq.km in 2001, which increased to 124–9252 during 2011. Whereas in low-burden states, the population density was in the range 13–1895 in 2001, which increased to 17–2169 persons per sq.km in 2011.

**Fig. 3.** Year-wise contribution of dengue cases in the three categories
The mean (and standard deviation, SD) of dengue cases is: 2852.33 (±4586.8) in high-burden states; 567.78 (±1344.7) in moderate-burden states; and 83.01 (±358.6) in low-burden states. Similarly, the mean of deaths due to dengue is: 8.94 (±13.20) in high-burden states; 1.00 (±2.40) in moderate-burden states; and 0.12 (±0.62) in low-burden states. The difference in the number of dengue cases and deaths is statistically significant (confirmed by ANOVA analysis) in all the three categories as shown in Table 2.

**Table 2. Statistical analysis of variants in the three categories**

<table>
<thead>
<tr>
<th>Variable</th>
<th>High-burden states</th>
<th>Moderate-burden states</th>
<th>Low-burden states</th>
<th>Test of significance (one-way ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dengue cases* Mean (SD)</td>
<td>2852.33 (4586.8)</td>
<td>567.78 (1344.7)</td>
<td>83.01 (358.6)</td>
<td>P value=0 F(calculated)=79.60 F(tabulated)=3.0 ANOVA test – highly significant at P&lt;0.0001</td>
</tr>
<tr>
<td>Dengue death Mean (SD)</td>
<td>8.94 (13.21)</td>
<td>1 (2.40)</td>
<td>0.12 (0.62)</td>
<td>P value=0 F(calculated)=84.45 F(tabulated)=3.01 ANOVA test – highly significant at P&lt;0.0001</td>
</tr>
<tr>
<td>Infection rate per 100 000 population</td>
<td>0.00–314.60</td>
<td>0.00–912.50</td>
<td>0.00–179.76</td>
<td>–</td>
</tr>
<tr>
<td>Population density in 2001</td>
<td>165–9340</td>
<td>100–7900</td>
<td>13–1895</td>
<td>–</td>
</tr>
<tr>
<td>Population density in 2011</td>
<td>201–11297</td>
<td>124–9252</td>
<td>17–2169</td>
<td>–</td>
</tr>
</tbody>
</table>
State-wise per cent contribution range of dengue cases and deaths, population contribution and population density (per sq.km) in 2001 and 2011 along with infection rate (per 100 000 population) during the study period are shown in Table 3.

**Table 3.** State-wise details of variants in the three categories

<table>
<thead>
<tr>
<th>State</th>
<th>Dengue cases range (%)</th>
<th>Dengue death range (%)</th>
<th>Population contribution (%)</th>
<th>Population density (per sq.km) 2001*</th>
<th>Population density (per sq.km) 2011*</th>
<th>Infection rate (per lakh population)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High-burden states</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Andhra Pradesh</td>
<td>0.03–7.66</td>
<td>0.00–11.46</td>
<td>3.67–7.08</td>
<td>277</td>
<td>308</td>
<td>0.03–10.61</td>
</tr>
<tr>
<td>Gujarat</td>
<td>1.95–15.84</td>
<td>0.00–10.24</td>
<td>4.97–5.31</td>
<td>258</td>
<td>308</td>
<td>0.06–26.92</td>
</tr>
<tr>
<td>Haryana</td>
<td>0.16–9.93</td>
<td>0.00–18.18</td>
<td>2.04–2.16</td>
<td>478</td>
<td>573</td>
<td>0.01–36.40</td>
</tr>
<tr>
<td>Karnataka</td>
<td>0.88–30.15</td>
<td>0.00–10.83</td>
<td>4.43–5.38</td>
<td>276</td>
<td>319</td>
<td>0.21–29.22</td>
</tr>
<tr>
<td>Kerala</td>
<td>0.00–27.80</td>
<td>0.00–31.63</td>
<td>2.57–3.40</td>
<td>819</td>
<td>859</td>
<td>0.00–58.11</td>
</tr>
<tr>
<td>Maharashtra</td>
<td>1.63–21.13</td>
<td>0.00–54.55</td>
<td>9.26–10.55</td>
<td>315</td>
<td>365</td>
<td>0.31–11.87</td>
</tr>
<tr>
<td>Odisha</td>
<td>0.00–15.86</td>
<td>0.00–19.53</td>
<td>3.34–3.93</td>
<td>236</td>
<td>269</td>
<td>0.00–18.95</td>
</tr>
<tr>
<td>Punjab</td>
<td>0.51–20.79</td>
<td>0.00–26.25</td>
<td>2.21–2.49</td>
<td>484</td>
<td>550</td>
<td>0.11–51.95</td>
</tr>
<tr>
<td>Rajasthan</td>
<td>0.00–43.92</td>
<td>0.00–66.04</td>
<td>4.47–6.50</td>
<td>165</td>
<td>201</td>
<td>0.00–17.04</td>
</tr>
<tr>
<td>Tamil Nadu</td>
<td>1.96–24.73</td>
<td>0.00–27.27</td>
<td>5.79–6.41</td>
<td>480</td>
<td>555</td>
<td>0.13–19.39</td>
</tr>
<tr>
<td>Telangana</td>
<td>0.00–7.04</td>
<td>0.00–4.22</td>
<td>2.62–2.90</td>
<td>**</td>
<td>**</td>
<td>1.74–8.47</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>0.00–11.64</td>
<td>0.00–17.14</td>
<td>14.73–17.67</td>
<td>690</td>
<td>828</td>
<td>0.00–4.52</td>
</tr>
<tr>
<td>West Bengal</td>
<td>0.00–53.19</td>
<td>0.00–21.66</td>
<td>7.18–8.49</td>
<td>903</td>
<td>1029</td>
<td>0.00–39.97</td>
</tr>
<tr>
<td>Delhi</td>
<td>2.34–27.69</td>
<td>0.00–27.27</td>
<td>1.28–1.54</td>
<td>9340</td>
<td>11297</td>
<td>0.35–88.15</td>
</tr>
<tr>
<td>Puducherry</td>
<td>0.00–6.98</td>
<td>0.00–2.15</td>
<td>0.09–0.11</td>
<td>2034</td>
<td>2598</td>
<td>0.00–314.60</td>
</tr>
<tr>
<td><strong>Moderate-burden states</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assam</td>
<td>0.00–5.97</td>
<td>0.00–2.07</td>
<td>2.49–2.77</td>
<td>340</td>
<td>397</td>
<td>0.00–18.57</td>
</tr>
<tr>
<td>Bihar</td>
<td>0.00–3.55</td>
<td>0.00–2.59</td>
<td>8.13–9.01</td>
<td>881</td>
<td>1102</td>
<td>0.00–5.50</td>
</tr>
<tr>
<td>Chhattisgarh</td>
<td>0.00–1.66</td>
<td>0.00–7.29</td>
<td>2.12–2.29</td>
<td>154</td>
<td>189</td>
<td>0.00–8.93</td>
</tr>
<tr>
<td>Goa</td>
<td>0.00–1.78</td>
<td>0.00–5.21</td>
<td>0.12–0.14</td>
<td>364</td>
<td>394</td>
<td>0.00–63.55</td>
</tr>
</tbody>
</table>
### Epidemiological stratification of dengue in India and strategic challenges

<table>
<thead>
<tr>
<th>State</th>
<th>Dengue cases range (%)</th>
<th>Dengue death range (%)</th>
<th>Population contribution (%)</th>
<th>Population density (per sq.km) 2001*</th>
<th>Population density (per sq.km) 2011*</th>
<th>Infection rate (per lakh population)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jammu and Kashmir</td>
<td>0.00–2.42</td>
<td>0.00–1.55</td>
<td>0.43–0.50</td>
<td>100</td>
<td>124</td>
<td>0.00–33.32</td>
</tr>
<tr>
<td>Madhya Pradesh</td>
<td>0.00–5.25</td>
<td>0.00–9.49</td>
<td>6.01–6.44</td>
<td>196</td>
<td>236</td>
<td>0.00–5.43</td>
</tr>
<tr>
<td>Uttarakhand</td>
<td>0.00–5.61</td>
<td>0.00–4.82</td>
<td>0.75–0.89</td>
<td>159</td>
<td>189</td>
<td>0.00–148.59</td>
</tr>
<tr>
<td>Chandigarh</td>
<td>0.00–1.79</td>
<td>0.00–0.83</td>
<td>0.8–0.9</td>
<td>7900</td>
<td>9252</td>
<td>0.00–113.27</td>
</tr>
<tr>
<td>D &amp; N Haveli</td>
<td>0.00–3.22</td>
<td>0.00–1.20</td>
<td>0.2–0.4</td>
<td>449</td>
<td>698</td>
<td>0.00–912.50</td>
</tr>
<tr>
<td><strong>Low-burden states</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arunachal Pradesh</td>
<td>0.00–1.93</td>
<td>0.00–0.45</td>
<td>0.10–0.12</td>
<td>13</td>
<td>17</td>
<td>0.00–133.86</td>
</tr>
<tr>
<td>Himachal Pradesh</td>
<td>0.00–3.56</td>
<td>0.00–4.07</td>
<td>0.45–0.48</td>
<td>109</td>
<td>123</td>
<td>0.00–7.65</td>
</tr>
<tr>
<td>Jharkhand</td>
<td>0.00–0.44</td>
<td>0.00–1.54</td>
<td>2.61–2.92</td>
<td>338</td>
<td>414</td>
<td>0.00–2.09</td>
</tr>
<tr>
<td>Manipur</td>
<td>0.00–1.17</td>
<td>0.00–1.45</td>
<td>0.21–0.26</td>
<td>97</td>
<td>122</td>
<td>0.00–12.60</td>
</tr>
<tr>
<td>Meghalaya</td>
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<td>0.00–0.83</td>
<td>0.20–0.26</td>
<td>103</td>
<td>132</td>
<td>0.00–5.24</td>
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<tr>
<td>Mizoram</td>
<td>0.00–0.45</td>
<td>0.00–0.00</td>
<td>0.08–0.10</td>
<td>42</td>
<td>52</td>
<td>0.00–49.49</td>
</tr>
<tr>
<td>Nagaland</td>
<td>0.00–0.28</td>
<td>0.00–0.45</td>
<td>0.15–0.20</td>
<td>120</td>
<td>119</td>
<td>0.00–18.65</td>
</tr>
<tr>
<td>Sikkim</td>
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<td>0.00–0.00</td>
<td>0.01–0.02</td>
<td>76</td>
<td>86</td>
<td>0.00–179.76</td>
</tr>
<tr>
<td>Tripura</td>
<td>0.00–0.8</td>
<td>0.00–0.00</td>
<td>0.30–0.34</td>
<td>305</td>
<td>350</td>
<td>0.00–3.14</td>
</tr>
<tr>
<td>A &amp; N Islands</td>
<td>0.00–0.34</td>
<td>0.00–0.00</td>
<td>0.03–0.05</td>
<td>43</td>
<td>46</td>
<td>0.00–36.13</td>
</tr>
<tr>
<td>Daman &amp; Diu</td>
<td>0.00–0.33</td>
<td>0.00–1.20</td>
<td>0.01–0.03</td>
<td>1413</td>
<td>2169</td>
<td>0.00–175.07</td>
</tr>
<tr>
<td>Lakshadweep</td>
<td>0.00–0.00</td>
<td>0.00–0.00</td>
<td>0.00–0.01</td>
<td>1895</td>
<td>2013</td>
<td>0.00–0.00</td>
</tr>
</tbody>
</table>

*Source: http://niti.gov.in/niti/content/population-density-sq-km

**Telangana was bifurcated from Andhra Pradesh on 2 June 2014

The impact of population expansion and urbanization in South-East Asia on dengue transmission has been well documented by studies done in Bangkok (52). The authors using mathematical modelling suggested that dengue virus resides in and spreads out of Bangkok and other urban centres, to the rest of the region. The spread occurred in three-yearly waves
of large outbreaks, each driven by a change in the predominant serotype of dengue virus. The authors also mentioned that with further expansion of the urban populations, dengue activity will remain at a level favourable for the continuation of cyclical epidemic activity, with each cycle being larger in magnitude. Similar observations of a super-annual cycle with a 3–4-year interval have been made in another study published in 2006 (53). During the present study, the peaks of dengue cases in all three categories are observed in different years during the past two decades and each peak was larger than the previous one.

The striking global geographical expansion and increased incidence of dengue coincided with urbanization. If the global trends of population growth, urbanization and globalization continue, there may be increase in the frequency, magnitude and severity of dengue (54). In the present study, there is a distinct correlation between the numbers of dengue cases reported by each category and the level of urbanization in each category. The high-burden states had the highest number of towns (74.41%) of the country, followed by much smaller numbers in moderate-burden (20.53%) and low-burden states (5.05%) (48).

The network of SSHs established for augmenting surveillance and diagnosis was strengthened from 110 hospitals in 2007 to 680 in 2019. However, increase in the number of SSHs does not reveal any association with the dengue cases reported during the study period as in high-burden states, there was a 4.9-fold increase in facilities from 2007 to 2019, 15.8-fold increase in moderate-burden states and 48-fold increase in low-burden states.

**Interventional issues and challenges**

In the evolution process from the first isolation of dengue virus in the country (during the Second World War (55) till its spatio-temporal spread to all states, challenges were different for each socio-geo-ecological setting. Hyper-endemicity of dengue is attributable to population growth and rapid urbanization, which are also the leading causes of the cyclic epidemiological pattern of dengue as shown in a study carried out in Bangkok (52).

Besides universal factors (6) responsible for occurrence and upsurge of dengue, the challenges increased manifold in the recent past. While stratifying the states on the basis of various factors, the challenges for prevention and control of dengue in different categories were also assessed and enlisted.

The majority of high-burden states are in the temperate zone having temperature and humidity suitable for vector mosquito supporting persistent and perennial transmission, thereby leading to an increased risk of dengue. Population density, population movement and transportation, which favour propagation of pathogens to newer sites, are the major challenges to contain the disease. With increased population growth and urbanization, demand for potable water is increasing (56,57) and availability of per capita water is decreasing. Scarcity of drinking water is considerable in this category of states, which compel
people for indiscriminate water storage – a critical factor contributing to Aedes breeding. In view of stable epidemiological features of this category, entomological surveillance through a robust system and timely analysis of the data are critical to prevent any possible upsurge or outbreak in the early stage. The disease trend has to be monitored through regular reports from the hospitals and laboratories engaged in dengue diagnosis and management. The surveillance system should be strong enough to capture dengue cases timely and accurately, predict impending upsurge/outbreaks and early initiation of necessary preventive measures. Overall, a holistic approach in a coordinated way involving stakeholders and the community at various levels is paramount.

The moderate-burden states have unstable dengue endemicity during the past two decades. The affected districts report a significant number of cases on a regular basis with few outbreaks. The remaining districts report either no case or negligible number of cases. The seasonal pattern of dengue transmission in inter-epidemic years in these states strongly suggests that surveillance needs to be enhanced and sustained even during the low transmission period. Major challenges in these states include their geography, climatic factors and population movement. An early warning system with both epidemiological and entomological surveillance that may be predictive of dengue transmission identifying risk-prone areas with possible upsurge in cases and to minimize the risk at an initial stage is important for this category of states.

In low-burden states, outbreaks in the past two decades are reported in few states confining to the affected districts only. Though outbreaks are rare, surveillance needs to be strengthened with entomological monitoring as these are epidemiologically vulnerable for any possible upsurge/outbreak. There is a need for sustained vector control activities regardless of transmission intensity. Laxity during inter-transmission periods is likely to compound entomological, epidemiological challenges and sporadic outbreaks.

This study is based on the data of 20 years, permitting analysis of inter-epidemic and epidemic transmission including typical seasonal variation. However, there are some limitations on the data as these include mostly the public sector and laboratory-based confirmed cases. It does not include data of probable cases, cases from the private sector which do not qualify the case definition, and those asymptomatic infections which are known to constitute 70–80% of cases.

There is a scope for studying the effect of climatic data on temperature, rainfall and associated entomological data, which may prove a strong indicator for assessment of transmission patterns in various categories.
Conclusion

Dengue is enlisted by WHO as one of the 10 threats to global health in 2019 (58). The efforts to control dengue face a number of challenges due to factors associated with speeding urbanization, environmental heterogeneity, shortage of resources and epidemiological priority in most of the states. Adequate epidemiological surveillance could serve as a robust basis to stratify resource allocation and identify the areas where programme interventions need to be focused.

Epidemiological risk analysis for dengue is useful for policy-makers and public health programme managers for allocation of resources in a more rational way and to address the requirements with an approach to deal with highly affected areas on priority. This stratification aims to decide differential strategies needed for each category in specific ways. Categorization is a dynamic process based on the spatio-temporal distribution of dengue at least for 5 years. The states may be included or excluded in and from a category in future based on the dengue burden.

Capturing of all suspected and confirmed cases through laboratory-based surveillance is essential to know the exact disease burden and for effective emergency response, which will help in keeping these states under respective categories. Passive surveillance systems are not suitable for monitoring dengue virus transmission. For all the categories, states were instructed to make dengue a notifiable disease, which is an essential step in this direction.

The significance of acceleration of efforts or a more focused approach is required for high-burden states; however, low- and moderate-burden states should not be complacent as little slackness in programme implementation at all levels may lead to outbreak/s depending on prevailing climatic conditions, rapid urbanization, improper solid waste management, water scarcity leading to storage, susceptible community, prevalence of vector and massive population movement. The criteria used in this study may be referred for stratification at the subnational level on the basis of disease burden and other factors to utilize resources in an effective manner through data-driven action plans and evidence-based interventions in priority areas.

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Conflict of interest

The authors declare no conflict of interest.
References


Impact of COVID-19 pandemic on prevention and control of dengue in Delhi, India

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Abstract

The COVID-19 pandemic has impacted human lives worldwide and cases are still increasing at a fast pace in 2020. In India, the global challenge of COVID-19 started showing its impact during late March 2020. Delhi being a metropolitan city with an international air terminal, the corona virus disease initiated in Delhi due to in-migration of infected persons from various parts of the world. Key strategies adopted to control transmission of COVID-19 included: lockdown, surveillance of tourists staying in different hotels of Delhi, health education and capacity building programmes for travellers, hotels, children, nodal teachers, resident welfare associations, and different stake holder departments. The sharing of human resources, which were invested in activities related to COVID-19, posed challenges for prevention and control of vector-borne diseases (VBDs) in Delhi. Extensive vector surveillance and their control received a setback, but with administrative support by mobilizing human resources from other departments, the public health department was able to initiate control measures for VBDs. Social media and other electronic media are key strategies for community sensitization during such pandemics. Lockdown can have both positive and negative impacts in dengue transmission. The use of newer vector tools such as Gravid Trap/Ovitrap will help in vector surveillance when resources are limited for vector control. This article focuses on the efforts made to address the challenges for prevention and control of VBDs during the COVID-19 pandemic and the recommendations for such situations in future when resources are limited and shared.

Keywords: COVID-19 pandemic; vector control; workforce; human resource; infected population; community sensitization

Introduction

The pandemic of COVID-19 has many lessons for public health professionals, clinicians and administrators across the globe, even for developed countries which have been worst affected. The pandemic started as Wuhan, Hubei province of China, communicated clustering of
pneumonia cases with no known aetiology to their WHO country office on 31 December 2019. These cases presented with respiratory symptoms leading to acute respiratory distress syndrome (ARDS) and respiratory failure. The world was unaware of the tragedy brewing in Wuhan and the causative organism was reported as novel corona virus in January 2020 and finally as SARS-CoV-2.

The Ministry of Health and Family Welfare, Government of India with timely intervention released the first “Travel Advisory” on 17 January 2020 as part of preventive measures. Subsequently, various guidelines were issued and travel restrictions were imposed. On 30 January 2020, an Indian student returning from Wuhan, China was reported as the first case of COVID-19 in India as an imported case. By 3 February 2020, two more Indian students who returned from China were tested positive for COVID-19.

In view of 118 000 COVID-19 cases and 4291 deaths reported across 114 countries, the Director General, World Health Organization declared COVID-19 as a global pandemic on 11 March 2020 (1).

COVID-19 in India

There is a rising trend of COVID-19 cases in India. A total of 1 435 453 confirmed COVID-19 cases and 32 771 deaths were reported in India till the morning of 26 July 2020; of these, 917 568 cases had recovered. The case fatality rate (CFR) was 2.28% (2). The Government of India imposed a nationwide lockdown on 24 March 2020 wherein no movement was allowed for 21 days. All offices except the health department and those of essential services were shut. The lockdown was further extended for two more weeks until 17 May. A major concern that remained was the transmission season of VBDs especially in the southern states of India. During 2017, India experienced an outbreak of dengue especially in southern states of India. Dengue transmission with its cyclical pattern posed a challenge in 2020.

COVID-19 in Delhi

Delhi reported its first case of COVID-19 on 2nd March 2020 and later Italian tourists with history of travel from Rajasthan and staying in a hotel of South Delhi were brought under surveillance and were found positive. A total of 130 606 confirmed COVID-19 cases and 3827 deaths were reported in Delhi till 26 July 2020. There were 11 904 active cases and more than 84% of cases had recovered. The CFR in Delhi was 2.9%, which was more than that reported for whole India. This can be attributed to better diagnostic facilities in Delhi. Most of the deaths reported were due to co morbid conditions. Total tests done in Delhi till that time were 946 777, i.e. 49 830 per million (3). This was also the transmission season of VBDs in Delhi especially dengue, which poses a major public health challenge. In the absence of COVID-19 pandemic, extensive control activities for dengue prevention would have been undertaken.
Prevention and control of COVID-19 and VBDs by civic bodies in Delhi

Measures for prevention and control of COVID-19 were initiated in Delhi by the three municipalities from February 2020. Key strategies included: surveillance of travellers staying in different hotels of Delhi, health education and capacity building programmes for travellers, hoteliers, children, nodal teachers, resident welfare associations (RWAs), stakeholder departments, etc. Stress was laid on hand hygiene/hand washing techniques and respiratory etiquettes. Masks were recommended for people with respiratory symptoms so that they did not infect people around them.

Delhi being an urban city, local civic bodies have the responsibilities for prevention and control of VBDs. There are five civic bodies in Delhi. Three municipalities, namely North Delhi Municipal Corporation, South Delhi Municipal Corporation and East Delhi Municipal Corporation, provide civic amenities and public health services in about 95% area of Delhi and the other two civic bodies cover 5% area. To accomplish this objective of prevention and control of COVID-19, the entire workforce of anti-malaria operations (responsible for the implementation of the National Vector Borne Disease Control Programme) was deployed.

Though all the four serotypes have been circulating in Delhi, DEN-1 has been reported to be the predominant. Any change in serotypes may lead to higher mortality (4).

Table 1. Different activities being performed by the staff of anti-malaria operations

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Category of workforce</th>
<th>Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anti-malaria officer</td>
<td>Coordination with stakeholders for workforce and equipment for sanitization; coordination with the sanitation department for removing biohazard waste from the houses of confirmed cases</td>
</tr>
<tr>
<td>2</td>
<td>Malaria inspector (malaria circle in-charge)</td>
<td>Supervision of the field staff engaged in sanitization activities; meetings with RWAs regarding preventive measures for COVID-19</td>
</tr>
<tr>
<td>3</td>
<td>Field workers</td>
<td>Sanitization with Knapsack pumps in houses of COVID-19-positive cases and cases under home isolation/quarantine; sanitization of containment zones</td>
</tr>
<tr>
<td>4</td>
<td>Domestic breeding checkers</td>
<td>Assist field workers in sanitization and awareness campaigns among residents for prevention and control of COVID-19</td>
</tr>
</tbody>
</table>

Table 1 show that the entire team responsible for prevention and control of VBDs was directed towards the prevention of COVID-19, thus bringing all measures for prevention and control of VBDs to a standstill. Supervisory officers including epidemiologists and entomologists were given the responsibility of supervision of various measures. Stakeholder departments, i.e. engineering and horticulture departments, were involved with their equipment to carry out sanitization operations supported by the above-mentioned staff.
Challenges in prevention and control of VBDs during COVID-19 pandemic in Delhi

(1) **Case surveillance and diagnostic dilemma:** Patients with fever may have overlapping signs and symptoms of dengue/chikungunya and COVID-19 and a provisional diagnosis of COVID-19 is made in most such patients. These patients are subjected to RT-PCR test for COVID-19. Dengue is also a self-limiting disease with varied clinical signs and symptoms and diagnosis can be missed, which lead to a diagnostic dilemma. Singapore reported two cases of COVID-19 who had false-positive dengue serology. Chikungunya also share similar clinical findings (5, 6, 7, and 8).

A case was reported in Thailand where a patient presented with clinical signs and symptoms of dengue with petechial rash. A primary diagnosis of dengue was made. Later, the patient developed respiratory distress and was tested positive for COVID-19. It was concluded that an early testing for local endemic conditions should be considered (1). A total of 34 dengue cases were reported in Delhi from 1 January to 25 July 2019, whereas 28 dengue cases were reported in 2020 for the same period. This reduction can be attributed to the decrease in the number of samples tested for dengue. Dengue wards were made operational in Delhi from the month of August. No case of concomitant infection of COVID-19 and dengue/chikungunya was reported (9).

**Fig. 1.** Month-wise VBD and COVID-19 cases during 2020 in Delhi

Fig. 1 shows the month-wise cases of dengue, malaria, chikungunya and COVID-19 notified in Delhi during 2020. Fewer cases were notified in 2020 compared to 2019 because most persons stayed indoors during the lockdown and there was no movement of individuals.
and the COVID 19 virus (SARS-CoV-2) Moreover, during the lockdown, the key message promoted was that citizens must do source reduction in and around their residence. After the unlock phase in June, cases of Vector Borne Diseases were reported.

**Fig. 2.** Age-wise distribution of dengue and COVID-19 cases in south zone, Delhi

Fig. 2 shows the age-wise distribution of dengue and COVID-19 cases in the south zone of Delhi. Fig. 2 shows that both diseases were prevalent in all age groups. The age group for dengue has been taken as average for the past 3 years because very few cases were reported in 2020. It was observed that dengue was more prevalent among children as well as those involved in outdoor activities, i.e. school, college and economic activities (job) compared to the elderly age group. The incidence of COVID-19 was reported less among children compared to adults. Both dengue and COVID-19 may have fatal outcome in elderly persons due to co morbid conditions.

If there was a shift in COVID cases towards the younger age group, or there was co infection of dengue and COVID-19, mortality might increase in Delhi. It was also predicted for low- and middle-income countries (LMICs) to have less mortality compared to high-income countries. Co infection with malaria or neglected tropical diseases (NTDs) might worsen the scenario in LMICs. He further reported that immuno modulation induced by malarial infection offers some protection against serious manifestations of respiratory viruses. It was observed in Kenya that children who were admitted in hospitals with diagnosis of influenza and malaria were less likely to experience respiratory distress than those with influenza alone (10). This can also be one of the reasons for less virulent COVID-19 infection in India as India is endemic for malaria.
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**Fig. 3.** Sex-wise distribution of cases of COVID-19 and dengue

Fig. 3 shows the sex-wise distribution of COVID-19 and dengue cases reported in the south zone. COVID-19 data show that 63% of cases were among males and 37% among females. Similarly, in dengue 56% of cases were reported among males and 44% among females. Male preponderance can be attributed to their outdoor activities.

(2) **Workforce:** The entire health workforce has been involved in prevention of COVID-19 since the first week of March 2020; thereby measures for prevention and control of VBDs were totally stopped. Table 1 shows the workforce involved in different activities. In addition to the lockdown in Delhi during March 2020, there was also absenteeism of staff due to non-availability of public transport, fear of going out and some areas falling under COVID-containment zones. Health workforce, i.e. field workers, domestic breeding checkers (DBCs) and even supervisory malaria inspectors who were the backbone for sanitization and anti larval measures, got infected with COVID-19 and had to be isolated for 14 days. Their contacts also had to be home quarantined and entire the malaria circle had to be closed. These factors affected prevention and control work. The absence of the second line of trained workforce to take over field activities also posed a challenge.

(3) **Vector surveillance:** DBCs are semi-skilled workers engaged to carry out domestic breeding checking and impart health education to the members of houses visited by them. During lockdown restrictions from 24 March 2020, DBCs could not conduct their assigned task and were deployed to assist in “hypo sprays” and impart education on prevention and control of COVID-19. Breeding checking was not carried out during this period because citizens restricted entries of outsiders into their houses due to fear of spread of COVID-19.

(4) **Community movements:** During the total lockdown, citizens were forced to stay indoors and there was limited movement. They took care of their houses and perhaps fought the mosquito nuisance on their own. This may be taken as a positive development. *Aedes* being an indoor breeder will not go out unless human bait is not available in the house, but in
the lockdown people were found in their respective houses, therefore it is most likely that Aedes mosquitoes might also not have moved much to nearby houses. On the other hand, the abandoned places where water collection was not monitored by DBCs as done before the lockdown might have increased mosquito densities in various places of Delhi. This makes residents vulnerable to mosquito bites. Temperature getting warmer from March onwards facilitated the shortening of larval life and increased adult emergence. The lockdown had closed almost all offices of the government and the private sector, as well as shops. There was no information whether water was stored in them and the status of water tanks as all these remained unchecked after March. In April 2020, temperatures of 25–32 °C, the favourable outdoor environment supported breeding of Aedes, which led to increased indoor presence of adults. Thereafter, sudden increase in temperature in May–June (38–40 °C), the outdoor sources might have been avoided by Aedes and all breeding shifted to indoors and in shady outdoor sites where temperatures were lower. All these remained unchecked during this period.

There were about 54% less home visits by DBCs in 2020 compared to 2019 due to the lockdown. However, data show 65% less breeding compared to 2019 due to limited data available after March 2020. Penalties for creating mosquitogenic conditions have also decreased by 65% (this is an enforcement action under Malaria Byelaws of DMC Act 1957 for encouraging or creating mosquitogenic conditions), which can be attributed to shutting of courts due to the lockdown. Under Malaria Byelaws, there is a provision of issuance of a legal notice or prosecution for which the offender has to present himself in a District Court for the offence.

**Fig. 4.** Comparative data on HI and CI for 2019 and 2020
Fig. 4 shows that the house index (HI) decreased in 2020 during the months of lockdown, i.e. April to May, as domestic breeding could not be checked. However, peridomestic breeding had high container index (CI) compared to 2019 outdoor breeding for the corresponding months from April to June in 2020.

(5) Vector control measures: All field workers responsible for carrying out anti larval and anti-adult operations were deputed for sanitization under COVID-19, which was a setback to anti larval measures resulting in high adult density. DBCs do source reduction and contain the container breeding by using temephos (sand granules), but these workers were involved in COVID-19-related surveys. Knapsack pumps were used to sanitize residences of confirmed COVID-19 cases with sodium hypochlorite solution. This solution being corrosive in nature has damaged metallic parts of these pumps, which need to be replaced.

(6) Support of research institutes: National level research institutes also faced a similar shortage of workforce and allocation of only COVID-19-related tasks, which affected research activities and support to implementing agencies.

(7) Community sensitization campaigns: Sensitization of community members regarding prevention and control of COVID-19 and VBDs was initiated by the supervisory staff and meetings were organized with RWAs. Following the lockdown, the same staff members were not welcomed in the offices of RWAs and had to restrict their meetings. However, efforts were made to sensitize people through media/social media.

(8) COVID care centre/isolation centres: To meet the demand of beds for COVID-19 patients, temporary hospitals were created and unoccupied apartments and hotels were converted into COVID-19 facilities. Patients admitted in these hospitals were already immune-compromised and the presence of another virus of dengue/chikungunya might have proved a catastrophe for them.

In Delhi, all hospitals are zero tolerance zones for mosquito breeding and to maintain these huge premises breeding-free is a major challenge for municipal authorities. Intermittent rains, solid waste, barrel dustbins in the open, condensation water from air conditioners collecting on the roadside and pavements, tyre marks and fire buckets, lack of workforce/infrastructure of hospital for vector control are some of the challenges for breeding control. Ae. aegypti is a predominant vector for transmission of dengue/chikungunya. A COVID care centre is situated in south Delhi near the ridge of Aravalli range and Ae. albopictus were captured in gravid traps, thereby posing a challenge because source reduction and fogging cannot be used for adult control for fear of deterioration of symptoms of COVID-19 patients.

Fig. 5 shows the monthly distribution of dengue cases reported in Delhi during 2015–2020. A major outbreak of dengue was reported in Delhi in 2015 (4) where more than 15 000 cases were reported. The next outbreak was reported in 2017 when 4726 cases were reported. Regular advisories have been issued by the Directorate of National Vector Borne Disease Control Programme for taking special measures for prevention and control of VBDs as there may be upsurge in cases in 2020.
In a small study in the North Delhi Municipal Corporation, clustering of COVID-19 cases were observed in those localities where clustering of dengue was observed in the past. This might be due to high congregation of human population in these localities. The simultaneous spread of COVID-19 and dengue might be severe due to co morbidities and immune-compromise situations. The entomological indices followed the pattern of the South Delhi Municipal Corporation, i.e. the HI decreased in the months of May onwards, whereas CI showed rising trends due to peridomestic checking.

WHO recommends that outbreak-prone diseases especially dengue should be monitored for clustering of cases, and all measures should be implemented to prevent an outbreak. In areas where dengue has established endemicity, diagnostics facilities should be maintained. Symptoms of both dengue and COVID-19 may overlap; hence health-care workers must promote warning signs and symptoms of dengue during community-based campaigns. During the pre-transmission and early transmission seasons, community programmes should aim to address preventive measures and during early transmission the objective should be to address source reduction and warning signs to avert mortality (11,12).

Week-wise house visits made and houses positivity. There was a setback in April 2020 following the complete lockdown since March-end. But later, there was a shift of strategy from house visits; surveillance was done in peridomestic areas and activities were continued (Fig. 6).
Innovative measures initiated to meet the challenges

In the absence of staff for VBDs control measures and unattended breeding sites, the challenge was accepted and innovative measures were initiated to combat the menace of VBDs.

**Additional workforce:** As the number of COVID-19 cases were raising in Delhi, the administrative authorities were requested for additional workforce to carry out sanitization. Support was provided with human resource of the horticulture department. In July, 100 additional personnel of the sanitation department were deputed to the south zone of South Delhi Municipal Corporation as the burden of cases was highest in the south zone.

**Equipment:** Sprinklers of the horticulture department were deployed for sanitization in the narrow lanes and jetting machines of the engineering department were used for sanitization of public places in large open areas. For this, 7% bleaching powder was used. Once guidelines were issued by WHO (12) regarding utility of outdoor sanitization, the frequency of the same was decreased. Knapsack pumps, which got damaged due to sodium hypochlorite, have been repaired and now being used for anti larval operations.

**Tackling adult density:** Adult density was reported in the month of April and the first round of indoor residual spray using 5% alpha-cypermethrin was done in cluster areas. Though Delhi being an urban area qualifies for anti larval operations, but narrow congested houses in clusters which lack civic amenities were covered with indoor residual spray. There was a
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decrease in adult density in June 2020. Indoor residual spray was also done in the COVID wards of the biggest COVID care centre in Delhi.

Project on breeding-free resident welfare associations (RWAs): A pilot project was launched with selected RWAs. They were requested to constitute a team of security guards, staff/labour of RWAs and two volunteer among the residents to go around the premises every week and carry out source reduction. Breeding indices were monitored by the zonal entomological team.

Gravid traps: Gravid traps using sticky strips were placed in COVID care centers for vector surveillance. Mixed *Aedes* and *Culex* breeding were reported. *Ae. albopictus* has been reported whereas in Delhi *Ae. aegypti* has been reported. Breeding was detected in rainwater collection in dustbins, which are mostly placed in the open. These dustbin drums were placed upside down as no use of them was found during the lockdown. Outdoor breeding sites were treated with Bti. Collaboration was established with a national level research institute for viral studies on pooled samples of mosquitos.

Community sensitization: Interpersonal communication and focus group discussion are some of the best strategies for behaviour change. There was restriction of community interaction during the lockdown. There are misconceptions and fears among the citizens that they might have COVID-19 transmission following an interaction with a health-care worker. A tool of mass communication especially social media was adopted. A key message during the lockdown was that the breeding habitat of *Aedes* are domestic and peridomestic settings and it rests indoor and since citizens are staying indoors, which makes them vulnerable to mosquito bite, therefore citizens themselves must check the breeding sites in and around their houses. The following other strategies were adopted:

(1) Advisories along with videos on preventive measures were shared with RWAs through social media.

(2) Auto-rickshaws with megaphone were hired and recorded messages on prevention of COVID-19 and mosquito breeding were played.

(3) Once the unlock process started, advisories were issued to all the administrative heads of government and private institutions under signature of the Commissioner South Delhi Municipal Corporation advising them on common breeding sites which were neglected during the lockdown and favoured vector breeding sites that may appear following Monsoon.

(4) Residents were advised to use personal protective measures.

(5) Enforcement action was taken against regular offenders i.e. who failed to take preventive and control measures and breeding was detected in their premises on repeated inspections and visits by Domestic Breeding Checkers or their supervisors.
**Construction sites:** During the lockdown, these were the abandoned and most neglected sites. These sites were placed under regular surveillance and contractors were asked to take source reduction and anti larval measures at their construction sites.

**Welfare of staff:** Staff members, who found it difficult to attend their duties during the lockdown or found it difficult to travel by public transport, were provided special curfew passes and accommodation was arranged in schools and hotels and the rent was paid by the department. All staff members were provided protective equipment including rain coats as they had got chemical burns on their back due to spilling of the hypo solution. Ayurvedic and homeopathic departments (Indian System of Medicine) of South Delhi Municipal Corporation provided immune-booster medicine to the entire staff.

In view of the ongoing COVID-19 pandemic, all essential health services have to continue especially the preventive measures for epidemic-prone diseases. The following strategies may be adopted and included in the guidelines for prevention and control of VBDs.

1. **Workforce planning:** Workforce of other stakeholder partner departments may be deployed to carry out source reduction, removal of waste articles and even anti larval measures. The second line workers should be identified for any future epidemics. This workforce should be given annual training on preventive measures for all public health emergencies.

2. **Case surveillance and management:** Coordination should be established with local hospitals and cases if tested negative for COVID must be tested for dengue/chikungunya. Linkages may be established with sentinel surveillance hospitals before the beginning of the transmission season. Diagnostic and case management facilities for VBDs must remain operational during any calamities. All medical staff responsible for providing treatment to dengue cases must be trained for critical signs of dengue and their management. Charts with protocols for identification of severity and management steps as per available resources and Guidelines prescribed by World Health Organization should be available for reference. Clustering of cases is common in Dengue as well as COVID 19 differential diagnosis must be made if patient does not respond to one treatment.

3. **Strengthening of vector surveillance:** Vector surveillance activities should be continued even during a COVID-19 outbreak. The skilled job of vector surveillance should be performed by the staff of the malaria department under supervision of an entomologist/biologist and key containers should be identified and targeted.

4. **Vector control:** As COVID-19 was reported in hot spot areas of dengue, hence these sites should be targeted intensively for vector control. Health-care workforce must use protective equipment while carrying out vector control measures in vulnerable areas or residence of COVID positive cases. In case of clustering of VBD cases, fogging may be used in hot spots as it immediately knocks down adult mosquitoes.
In cluster areas, Indoor Residual Spray applied in mosquito resting places will help in preventing VBDs. During lock-down, households may be emphasised on need of clearing of stagnant water in and around their houses and all water storage containers must be covered. (14). Gravid sticky traps will act as surveillance tool and control measures for adult mosquitoes.

(5) **Stakeholder departments:** Support of the building department/engineering department may be taken to target construction sites for source reduction. The contractor/developer must be asked to take anti larval measures at construction sites or he should pay charges to municipalities for carrying out vector control measures at construction sites. The education department may be asked to send appropriate messages to school children on social media to check their houses. Similarly, other partner departments may be provided action points pertaining to their role in prevention of mosquito breeding.

(6) **Community sensitization:** Mass media/social media may be used for reaching community members under a lockdown and citizens may be explained the need for checking breeding sites in domestic and peridomestic areas and source reduction. Sending simple videos and messages through brand ambassadors is an effective strategy for motivating community members.

**Conclusion**

The entire world is facing the COVID-19 pandemic. Dengue is endemic in more than 100 countries. The presence of dual infection of COVID-19 virus and dengue virus in a patient may increase both morbidity and mortality. In 2020, large numbers of dengue cases were reported in many South-East Asian countries, Singapore, etc. In Delhi, a declining trend of COVID-19 cases has been observed but the transmission season of dengue/chikungunya has started. Following the rains there was a spread of breeding from domestic to paridomestic areas and sites are going to multiply. A key strategy is extensive vector surveillance and their control. This activity should be advocated to residents as they are forced to stay indoors due to the lockdown. Social media and other electronic media may be used for community sensitization. Use of a newer tool, i.e. gravid trap/ovitrap should be used when workforce deployment is compromised either due to shortage or affected due to disease outbreak to help in vector surveillance and resource mobilization for vector control.

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belief in the authors to tackle both public health challenges. The authors are also grateful to the Municipal Health Officer, Additional Municipal Health Officers for providing logistic support and my core personal office staff for their encouragement and work at odd hours and odd moments.

References


Impact of COVID-19 pandemic on prevention and control of dengue in Delhi, India

**Photo gallery**
(Challenges and Measures for prevention and control of VBDs at a COVID Care Centre)

- Collection of condensation water from air conditioners and wash basins
- Tyre marks filled with rainwater had *Culex* breeding
- *Aedes* breeding in rainwater collection in fire-safety buckets
- Gravid trap for surveillance
- Anti larval spray using Bti
- Indoor residual spray inside the ward of the COVID Care Centre
| Awareness campaign for prevention of COVID-19 | Awareness campaign for prevention of VBDs | Distribution of handbills by a domestic breeding checker (DBC) |
| Field workers at the Tera Panthi Isolation Centre for sanitization | Sanitization with a drone | Sanitization by a field worker at a COVID-19-positive house |
| Sanitization at a containment zone | Largest COVID-19 Care Centre | Food distribution to homeless |
| Breeding checking at abandoned construction sites | Breeding checking in an uncovered water storage tank | Breeding checking in a cemented tank |
Epidemiological and entomological investigation of an outbreak of dengue fever in Durg district of Chhattisgarh, India

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b National Centre for Disease Control, Jagadalpur, Chhattisgarh 494001, India
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d Centre for Medical Entomology &Vector Management, National Centre for Disease Control, Delhi 110054, India

Abstract

Dengue fever is one of the serious public health problems worldwide including India. Almost all the states of India are now endemic for dengue infection. Durg district of Chhattisgarh experienced an outbreak of dengue fever during August 2018. Epidemiological and entomological investigations were conducted to know the risk factors responsible for the dengue outbreak and recommended control measures. Age- and sex-wise analysis revealed that the most affected age group was 16–25 years and males were more affected than females (1.27:1). The state health authority reported the prevalence of DEN-3 serotype. Entomological indices, such as the house index (HI), container index (CI), breteau index (BI) and pupal index (PI), were found above the critical level in all the surveyed areas, which were in the range: HI 45–78, CI 9.8–20.2, BI 73.3–146.3 and PI 33.3–88.8. The highest breeding preference was observed in plastic storages (41.0%) followed by cement tanks (26.0%), others (unused plastic disposable cups, plates, etc.), tyres (8%), earthen pots (4%) and coolers (1.0%). Adult survey revealed the prevalence Aedes aegypti, Aedes albopictus and Aedes vittatus. High larval indices and the presence of two known vector species of dengue reflect the need for continuous and intensive antilarval and anti-adult operations during outbreaks followed by weekly entomological surveys for the dengue vector to prevent further outbreaks. Information, education and communication (IEC) and behaviour change communication (BCC) activities should be introduced simultaneously among the community.

Keywords: Aedes species; breeding sources; house index; breteau index; adult density; dengue fever.

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Introduction

Dengue fever (DF) is an acute febrile illness caused by flavivirus, family Flaviviridae. DF and dengue haemorrhagic fever (DHF) are the most important public health problems in tropical, subtropical and temperate regions of the world (1). The disease is now endemic worldwide covering over 100 countries with 3.9 billion people presently at risk of transmission of dengue, with Asia alone representing ~70% of the global burden of disease (2). The first epidemic of dengue-like illness in India occurred in Chennai in 1780 and the first virologically proved epidemic of DF occurred in Kolkata and Eastern Coast of India in 1963–64 (3,4). Dengue is caused by one of the four closely related serotypes (DEN-1, DEN-2, DEN-3 and DEN-4). DF is a self-limiting disease but some patients may develop DHF and dengue shock syndrome (DSS) depending on re-infection with serotypes (1,5,6,7).

Dengue virus is transmitted by the bite of infected female *Aedes* mosquitoes mainly *Ae. aegypti* and *Ae. albopictus*. Dengue is widespread in the Indian subcontinent, with risk factors influenced by local spatial variations of rainfall, temperature, relative humidity, degree of urbanization and suboptimal vector control services in urban areas. Its impact on economy, particularly in tropical and subtropical countries, has been well documented (6). For prevention and control of dengue, various approaches under integrated vector management (IVM) have yielded fruitful results. Among the three components of dengue surveillance, namely, disease surveillance, vector surveillance, and monitoring of environmental and social risks; entomological surveillance is an important tool used to determine changes in the geographical distribution and vector prevalence, evaluate control measures and facilitate appropriate and timely decisions regarding interventions.

The objectives of the study during investigation were: (i) to review and assess the situation of dengue outbreak in Durg district; (ii) to conduct an epidemiological and entomological survey in some of the affected areas; and (iii) to recommend remedial measures to overcome the current outbreak based on findings.

Material and methods

Study areas

Durg district is situated on the east bank of river Shivnath. The district is situated in the southern part of Chhattisgarh 40 km from Raipur (capital of Chhattisgarh). It is one of the densely populated districts of the state (Fig. 1). The area of Durg district is 2238.36 sq.km. This district is 317 metres above mean sea level. Durg lies between 20°54’ and 21°32’ north latitude and 81°10’ and 81°36’ east longitude. Climate is tropical type, annual average rainfall is 1052 mm, and July is the month of highest rainfall. It has an average annual temperature in
the range of 14–32 °C. As per the Census 2011 (provisional), the population of the district is 1,721,726 (617,184 rural and 1,104,542 urban), with a majority of the population residing in urban areas. According to the Census 2011, the population density of Durg district was 92 people per sq.km.

**Fig. 1.** Map showing outbreak location of Durg district and distribution of dengue cases

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**Epidemiological investigation**

The details of the affected areas and outbreak were collected from district health authorities, medical and paramedical staff through meetings and discussions. The number of confirmed cases in outpatient department (OPD) and inpatient department (IPD) were collected from hospital records. The details of control measures undertaken were also taken into account. Interviews with some of the patients were also conducted about the clinical presentation. Data for the past 5 years of dengue cases were obtained to assess the current situation of outbreak.

**Entomological investigation**

Entomological investigation was carried out as per the standard guidelines of World Health Organization (WHO) and National Vector Borne Disease Control Programme, Delhi. The team observed the sanitation practices, water-storage habits, potential breeding sites for the mosquitoes. Wet containers were searched on priority for the presence of *Aedes* larvae and pupae both in domestic and peridomestic areas to determine larval indices. Larvae were
identified and kept for adult emergence to identify the species. Adult mosquito collection was also done in early morning hours with the help of the Torch and Suction tube method to calculate the vector density.

RESULTS

Epidemiological investigation

Table 1 shows that male preponderance was observed in dengue cases, i.e., 273 (56.06%) male patients and 214 (43.94%) female patients. A majority of the cases were in the age group of 16–25 years followed by 6–15 years and 26–35 years. This shows that the school-age children and persons in the age group of 16–35 years were more affected. Year-wise data (Fig. 2) show that dengue cases have been reported in Durg district since the year 2012, but a large number of dengue cases (589) with seven deaths were reported till August 2018. The maximum number of dengue patients admitted to various hospitals of Durg district were observed from 6 to 16 August 2018 (Fig. 3).

During the household survey, the persons recovered were also interviewed. A majority of the patients had fever, headache and vomiting as the presenting symptoms as reported by the physicians. Of the 45 persons interviewed, only two patients had arthralgia and one patient had retro-orbital pain. During hospital visits, it was observed that few patients had sudden drop in their platelet count. As per the record, a 19-year-old female was the index case of the outbreak. She was diagnosed for dengue on 30 June 2018 and had a history of travel to Vijayanagaram of Andhra Pradesh.

Table 1. Age- and gender-wise distribution of dengue cases

<table>
<thead>
<tr>
<th>Age group</th>
<th>Gender</th>
<th>Female n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–5</td>
<td>22 (4.52)</td>
<td>20 (4.12)</td>
<td>42 (08.62)</td>
</tr>
<tr>
<td>6–15</td>
<td>80 (16.43)</td>
<td>51 (10.47)</td>
<td>131 (26.90)</td>
</tr>
<tr>
<td>16–25</td>
<td>83 (17.04)</td>
<td>64 (13.14)</td>
<td>147 (30.18)</td>
</tr>
<tr>
<td>26–35</td>
<td>44 (09.03)</td>
<td>49 (10.06)</td>
<td>93 (19.10)</td>
</tr>
<tr>
<td>36–50</td>
<td>26 (05.34)</td>
<td>21 (04.31)</td>
<td>47 (09.65)</td>
</tr>
<tr>
<td>≥51</td>
<td>18 (03.70)</td>
<td>09 (01.84)</td>
<td>27 (05.54)</td>
</tr>
<tr>
<td>Total</td>
<td>273 (56.06)</td>
<td>214 (43.94)</td>
<td>487</td>
</tr>
</tbody>
</table>
Fig. 2. Year-wise reported dengue cases and deaths in Durg district

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of dengue cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2013</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>2014</td>
<td>132</td>
<td>2</td>
</tr>
<tr>
<td>2015</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2016</td>
<td>56</td>
<td>2</td>
</tr>
<tr>
<td>2017</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2018</td>
<td>589</td>
<td>7</td>
</tr>
</tbody>
</table>

Fig. 3. Date-wise number of admitted dengue cases in various hospitals of Durg

Entomological observations

An entomological survey was carried out at Durg district where the outbreak of dengue was ongoing. For the *Aedes* larval survey in five localities, a total of 259 houses were searched. Of the 259 houses surveyed, 163 were found positive for *Aedes* breeding and of the 1736 containers searched, 271 were detected with *Aedes* breeding. *Aedes* larval indices were
found to be above the critical level indicating the intensive transmission of dengue (Table 2). *Aedes* indices such as HI, BI and PI were found above the critical level in all the visited areas (Table 2).

**Table 2.** Results of *Aedes* larval survey carried out at Durg district

<table>
<thead>
<tr>
<th>Locality</th>
<th>House index (HI)</th>
<th>Container index (CI)</th>
<th>Breteau index (BI)</th>
<th>Pupal index (PI)</th>
<th>Species identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shankar Nagar Chhavani</td>
<td>63.5</td>
<td>14.0</td>
<td>98.0</td>
<td>57.7</td>
<td><em>Ae. aegypti</em> <em>Ae. vittatus</em></td>
</tr>
<tr>
<td>Balaji Nagar</td>
<td>78.0</td>
<td>18.2</td>
<td>146.3</td>
<td>59.5</td>
<td><em>Ae. aegypti</em> <em>Ae. vittatus</em></td>
</tr>
<tr>
<td>Bapu Nagar</td>
<td>71.6</td>
<td>20.2</td>
<td>95.0</td>
<td>58.3</td>
<td><em>Ae. aegypti</em> <em>Ae. vittatus</em></td>
</tr>
<tr>
<td>Satnai Mohalla, Chhavani</td>
<td>45.0</td>
<td>9.8</td>
<td>73.3</td>
<td>33.3</td>
<td><em>Ae. aegypti</em> <em>Ae. vittatus</em></td>
</tr>
<tr>
<td>Utai (Semi-urban)</td>
<td>62.2</td>
<td>18.1</td>
<td>131.1</td>
<td>88.8</td>
<td><em>Ae. albopictus</em> <em>Ae. vittatus</em></td>
</tr>
</tbody>
</table>

The breeding preference of *Aedes* mosquito in the area was maximum to plastic containers (41.0%) followed by cement tanks (26.0%), tyres (8%), earthen pots (4%), coolers (1.0%) and “other” including unused plastic disposable cups, plates, etc. (Fig. 4). Coolers showed minimum breeding preference because most of the coolers were found dry due to the humid environment.

**Fig. 4.** Bar chart showing *Aedes* mosquito container breeding preference
Of the nine mosquito species collected, *Culex quinquefasciatus* (50%) was found in high percentage followed by *Armigeres* spp. (17%), *Anopheles subpictus* (14%), *Ae. vittatus* (6%) and *An. vagus* (5%). The primary vector of dengue *Ae. aegypti* (3%) was found in fewer numbers followed by *Ae. albopictus* (2%) (Fig. 5).

**Fig. 5.** Results of adult mosquito collection done in Durg district

<table>
<thead>
<tr>
<th>Mosquito Species</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ae. vittatus</em></td>
<td>50%</td>
</tr>
<tr>
<td><em>Cx. quinquefasciatus</em></td>
<td>17%</td>
</tr>
<tr>
<td><em>An. culicifacies</em></td>
<td>14%</td>
</tr>
<tr>
<td><em>Cx. vishnui</em></td>
<td>14%</td>
</tr>
<tr>
<td><em>Ae. aegypti</em></td>
<td>6%</td>
</tr>
<tr>
<td><em>An. subpictus</em></td>
<td>6%</td>
</tr>
<tr>
<td><em>Armigeres sp.</em></td>
<td>5%</td>
</tr>
<tr>
<td><em>An. vagus</em></td>
<td>5%</td>
</tr>
<tr>
<td><em>Ae. albopictus</em></td>
<td>2%</td>
</tr>
</tbody>
</table>

**Discussion**

This study covered dengue-affected areas of Durg district, which reported a large number of dengue cases with many deaths during August 2018. Sporadic dengue cases have also been reported from this area since 2012. The current outbreak started in the month of July 2018 with a single case and increased to 589 in the month of August. Males were more affected than females (1.27:1). The most affected age group was 16–25 years (30.18%), which is similar to that reported by Agarwal et al. (1999) (5), who recorded the age group of 11–30 years as the most affected in their study of 206 patients.

Due to the intermittent supply of water, people had to store the water for routine domestic purposes, which resulted in abundance of favourable breeding sites for *Aedes* mosquitoes. High larval indices showed that the area was vulnerable to dengue transmission as has been documented by Hadisoemarto and Castro (2013) (7). The breeding preference to plastic containers further supplements that the water-storing habit has contributed to increased breeding potential in the area. A large number of field workers were deployed for source reduction activities.
Besides antilarval measures such as source reduction and application of temephos, intensive fogging operations were also undertaken both indoors and outdoors. Indoor fogging was done with pyrethrum and outdoor with cyphenothrin, which reduces vector density. The outbreak containment process was facilitated by a team from National Centre for Diseases Control Delhi, which provided training to all the field workers and a special awareness meeting was held for school students to carry out source reduction activities in their school campus as well as in their houses. However, such activities on creating awareness need to be undertaken at regular intervals with an emphasis on observing one dry day every week by all.

Conclusion

An outbreak of dengue was confirmed in Mouhari, Marauda, Bhilai subdistrict, Durg district. The index case had a history of travel. The risk factors associated with the dengue fever outbreak may be attributed to the lack of public awareness regarding breeding sites of dengue vector and its association with water storage in houses because of an intermittent supply of water. The BI and PI were found above the critical level in all the surveyed areas.

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References


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Dengue Bulletin welcomes all original research papers, short notes, review articles, letters to the Editor and book reviews which have a direct or indirect bearing on dengue fever/dengue haemorrhagic fever prevention and control, including case management. Papers should not contain any political statement or reference.

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Figures and tables (Arabic numerals), with appropriate captions and titles, should be included on separate pages, numbered consecutively, and included at the end of the text with instructions as to where they belong. Abbreviations should be avoided or explained at the first mention. Graphs or figures should be clearly drawn and properly labelled, preferably using MS Excel, and all data clearly identified.
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