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A Survey and Investigation of an *Aedes* sp. Infested Multi-chambered Sewage Tank Supports the Concept of a Novel *Aedes* sp. Ecosystem

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Abstract

Aedes sp. Mosquito-borne diseases are causing community health problems, conventional studies are not getting proper controlling measures. In search of some innovative pathway, a ground-breaking hypothesis has been framed on a new ecosystem of *Aedes* sp. in the storage water of septic tanks. A thorough survey and studies have been conducted on the *Aedes* sp. infested water reserve of multi-chambered septic tank. The data has been computed through the larval survey technique and reflected that all the parameters like House Index /Container Index /and Breteau Index, are very high, its mean value is 72 for all 3 parameters, in West Bengal and Jharkhand area HI, CI, and BI are 68 (mean value) in all 3 parameters and overall value of both West Bengal and Jharkhand area are 70. These results are supportive and also contradictory to the views of different authors. So, all the findings concluded that the water reserve of a septic tank filled with 'so-called dirty water' can be considered as an alternative breading site of *Aedes* sp. leading to a condition of "New Eco-System of *Aedes* sp. Mosquito".

***Key words*:** *Aedes* sp., Infested septic tank, New eco-system, Tank survey, Clear and clean water

The basic idea of this study is to establish the concept that *Aedes* sp., (Diptera: Culicidae) medically important mosquito, are exploiting the water reserve of the Septic Tank as their alternative breeding ground for a new ecosystem. As per "WHO Comprehensive Guidelines for Prevention and Control of Dengue and Dengue Hemorrhagic Fever – Page No-71: Throughout most of South-East Asia, *Ae. aegypti oviposits* almost entirely in domestic and man-made water receptacles. These include a multitude of receptacles found in and around urban environments (households, construction sites, and factories) such as water-storage jars, saucers on which flowerpots rest, flower vases, cement baths, foot baths, wooden and metal barrels, metal cisterns, discarded tires, bottles, tin cans, polystyrene containers, plastic cups, discarded wet-cell batteries, glass containers associated with spirit houses (shrines), drainpipes and ant-traps in which the legs of cupboards and tables are often rested" [1]. Study reveled in 'Down to Earth' – Mosquito Matter - "The blood-sucking pest is altering itself with changing environment. But studies on it are few and far between Dengue-causing *Aedes aegypti* mosquito breeds in stagnant water containers such as drums, buckets, pots, and flower vases" [2]. Regarding Breading Sites and Characteristics of *Aedes* sp., the following views are presented by renowned authors "Dengue is one of the urban diseases and keeps increasing annually. Two mosquito species responsible for dengue transmission in Malaysia are *Aedes aegypti* and *Aedes albopictus*. Both of the species are commonly found near human communities. *Aedes* species can be found easily in natural and artificial containers with clear and clean water. Some of the preferred breeding sites are containers such as ant traps, earthen jars, flower pots, drums, concrete tanks, coconut shells, and discarded tires" [3-4].

Aedes aegypti bred in all types of water-holding containers even if it prefers some of the containers to others. Most of the mosquito larvae were collected from containers containing rainwater [5]. "In urban surroundings, mosquitos are more prone to utilize water-holding containers as immature developmental stages. Almost all mosquito species would prefer to oviposit in a less lighting area or shaded. Where in that particular area the temperatures are lower compared to the area that is exposed to direct sunlight, but there are still many other factors that take into account female mosquito ovipositional preferences places. One of the critical parameters of the aquatic habitats for mosquitoes is the chemical characteristics that can influence the survival rate of the mosquitoes. The conditions that would lead to mosquito infestation such as stored water in the container for an extended period, extended rainfall during the last rainy season, and ambient relative humidity and temperature" [5]. Since long past different types of investigational, survey, and analytical study, works are going on in full swing but no effective result has yet been reached for taking any preventive measures against *Aedes* sp. borne diseases rather in some cases the result is confusing [6] and not also encouraging. As per WHO "Before 1970, only nine countries had experienced severe dengue epidemics. The disease is now endemic in more than 100 countries." [7]. Since

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the criteria for a suitable breeding site for *Aedes* sp. mosquitoes are met by the water storage in the septic tank, a decision has been made to conduct a survey on the frequency of *Aedes* sp. infestation in septic tanks. This survey is part of a comprehensive doctoral thesis aimed at testing a hypothesis about a new ecosystem involving *Aedes* sp. mosquitoes. Other parameters related to larval growth have been studied independently. It has been observed that there is a significant lack of understanding of the Life Cycle of *Aedes* sp. mosquitoes, particularly concerning their ability to adapt and thrive in different environments. Therefore, addressing this pressing issue requires a comprehensive and novel approach that encompasses various nonconventional aspects. Hence, this study aims to examine the prevalence of *Aedes* sp. infested septic tanks and determine the mosquito genus responsible for producing significant public health issues.

MATERIALS AND METHODS

Study area

The survey and study conducted in some regions of Jharkhand and West Bengal, two neighboring states in India, aimed to determine the prevalence of *Aedes* sp. by examining the infestation indices of positive house and positive container, namely the Septic Tank (ST). The objective was to establish the generalization of the study findings. The following are the designated sample areas.

Sampling

A total of 50 sample sites were chosen to conduct a survey, with sites 1-5 located in West Bengal and sites 6-10 located in Jharkhand. A total of 5 samples were gathered from each respective region (Table 1).

Table 1 List of sampling areas with their geographical location

S. No	Sampling area	Coordinate of the area	State
1.	Asansol	23.6889^{0}N, 86.96610E	West Bengal
2.	Burnpur	23.6445^{0}N, 86.9326^{0}E	
3.	Durgapur	23.5204^{0}N, 87.3119^{0}E.	
4.	Pandabeswar	23.7097^{0}N, 87.2768^{0}E	
5.	Purulia	23.3322^{0}N, 87.3616^{0}E	
6.	Bokaro	23.6693^{0}N, 86.1511^{0}E	Jharkhand
7.	Dhanbad	23.7957^{0}N, 86.4304^{0}E	
8.	Jamshedpur	22.8046^{0}N, 86.2029^{0}E	
9.	Jharia	23.7426^{0}N, 86.4111^{0}E	
10.	Ranchi	23.3441^{0}N, 85.3096^{0}E	

The current investigation focused on *Aedes* sp. and was carried out in several dengue-prone locations, particularly urban and semi-urban areas where the presence of multi-chambered septic tanks is essential and common. This survey has partially adhered to the approach outlined by the Getachew *et al*. [5] study.

A specially built mosquito capturing cage, comprised of a 1.2-millimeter mesh size mosquito net, was installed at specific locations (Fig 1) for a minimum of 48 hours. The installation technique is somewhat similar to installing a plankton net at the source point of flowing water. This study employed a combined approach of insect and zooplankton collection methods, as described in the Zooplankton Methodology Manual [8]. The survey was carried out between September 2020 and August 2022 to investigate septic tank infestation. Each septic tank is surveyed once for 48 hours to determine if it is infested with *Aedes* sp. mosquitoes. This is done by checking for the presence of adult *Aedes* sp. mosquitoes trapped in the trapping cage (Fig 1-2).

Fig 1 Mosquito trapping cage (new designed) fitted at the outlet port of the septic tank for trapping adult mosquito, hundreds of *Aedes* Sp. are trapped in the cage after 48 hrs of fitting (distance view)

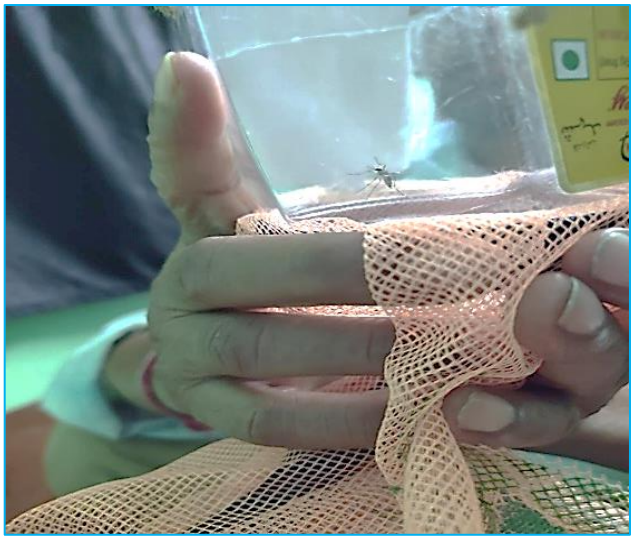

Fig 2 Photograph of trapped single *Adult* sp., separated for identification (close view)

After collection field identification of Genus was done by using the key [8-9]. Some adult mosquitoes were preserved by following a partially modified method [4-5].

RESULTS AND DISCUSSION

The survey results have been documented in a tabular format in (Table 2), displaying the survey report of areas that are either positive (infested by *Aedes* sp.) or negative (not infected by *Aedes* sp.). Waste management system for sewage.

The current study has adhered to a modified version of the standard technique for the Larval Survey, as outlined by the World Health Organization in 2016. This protocol is used to calculate infestation indices for Houses and containers given in the text. There are a total of 50 survey premises, with 25 of them located in West Bengal, their values for HI, CI, and BI are all computed to be 72 (Table 3). The number of premises belonging to Jharkhand is also 25, the calculated values for House Index (HI), Container Index (CI), and Breteau Index (BI) are all 68. The infestation indices are determined by calculating

the proportion of dwellings or containers that test positive for infestation, as stated by the World Health Organization in 2016. The survey data on infested septic tanks has been analyzed using modified larval survey techniques (Fig 3) including the House Index (HI) calculated using equation (1), the Container [Septic Tank- (ST)] Index (CI) calculated using equation (2) (where the septic tank is considered as a man-made artificial container), and the Breteau Index (BI) calculated using equation (3), as described above. The mean values for the House Index (HI), Container Index (CI), and Breteau Index (BI) for all 50 examined locations are 70, 70, and 70, respectively. (Table 3) provides a comprehensive and detailed representation of these values. The results of the present study indicate that all the factors, namely HI (House Index), CI (Container Index), and BI (Breteau Index), of the larval survey indices are significantly elevated.

Table 2 Shows the survey report of positive and negative septic tank

Site of West Bengal						
Asansol	23.6889^{0}N, 86.96610E	Y	Y	Y	N	Y
Burnpur	23.6445^{0}N, 86.9326^{0}E	Y	Y	N	N	Y
Durgaur	23.5204^{0}N, 87.3119^{0}E.	N	Y	Y	Y	Y
Pandabeswar	23.7097^{0}N, 87.2768^{0}E	Y	Y	N	N	Y
Purulia	23.3322^{0}N, 87.3616^{0}E	N	Y	Y	Y	Y
Site of Jharkhand						
Bokaro	23.6693^{0}N, 86.1511^{0}E	Y	N	Y	Y	N
Dhanbad	23.7957^{0}N, 86.4304^{0}E	N	Y	Y	Y	Y
Jamshedpur	22.8046^{0}N, 86.2029^{0}E	Y	Y	N	Y	Y
Jharia	23.7426^{0}N, 86.4111^{0}E	N	Y	Y	N	Y
Ranchi	23.3441^{0}N, 85.3096^{0}E	N	Y	Y	Y	Y

YES = Y (Positive Tank), No = N (Negative Tank)

Larval survey computation formulas

A. House / premise index (HI)

$$HI = \frac{\text{No. of Houses (+) for } \textit{Aedes} \text{ sp}}{\text{No. of houses inspected}} \times 100 \ldots\ldots\ldots (1)$$

B. Container index (CI)

$$CI = \frac{\text{No. of containers (+) for } \textit{Aedes} \text{ sp}}{\text{No. of containers inspected}} \times 100 \ldots\ldots\ldots (2)$$

C. Breteau index (BI)

$$BI = \frac{\text{No. of positive containers}}{\text{Total No. of houses inspected}} \times 100 \ldots\ldots\ldots (3)$$

Table 3 Shows area-wise and total indices of different parameters of the survey

S. No.	Survey area West Bengal / Jharkhand	Total house	Infested house	Total container (ST)	Infested container (ST)	HI	CI	BI
1	Asansol	5	4	5	4	80	80	80
2	Burnpur	5	3	5	3	60	60	60
3	Durgapur	5	4	5	4	80	80	80
4	Pandabeswar	5	3	5	3	60	60	60
5	Purulia	5	4	5	4	80	80	80
	Total / Average	25	18	25	18	72	72	72
6	Bokaro	5	3	5	3	60	60	60
7	Dhanbad	5	4	5	4	80	80	80
8	Jamshedpur	5	3	5	3	60	60	60
9	Jharia	5	3	5	3	60	60	60
10	Ranchi	5	4	5	4	80	80	80
	Total/ Average	25	17	25	17	68	68	68
	Grand total and average	50	35	50	35	70	70	70

House Index= HI, Container (ST) Index= CI, Breteau Index = BI

Vector indices have great correlation with *Aedes sp.* Mosquito abundance and dengue epidemics, but some authors are not getting results in favour of this theory. "Association between dengue cases and larval indices, BI/CI/ HI/ OI, and meteorological parameters was not significant. Migration of mosquitoes and patients could be considered as possible factors affecting the absence of a significant relationship" [6]. It is supporting the present study regarding alternative *Aedes* sp. ecosystem, besides conventional parameters there is alternate and neglected factors like septic tank water reserve as artificial container which may cause abundance of dengue epidemic despite low and even absence of conventional HI /CI / BI load. Moreover, the good outcomes provide evidence to support Sood's assertion in 2016 [2] that this mosquito adapts its habitat preferences in response to environmental changes. However, it is important to note that there is a scarcity of studies that confirm these findings. On the other hand, the present results are also very much contradictory to the findings of [5], in another similar type of study it was found that *Aedes* sp. preferred artificial containers filled with non-dirty clean, and clear water as their breeding ground [4].

CONCLUSION

The results obtained from both positive and negative approaches validate the current study. The study's findings demonstrate that the water reserve of the multi-chambered septic tank can be regarded as a highly successful alternate breeding habitat for *Aedes* sp. mosquitoes. This phenomenon may be attributed to the remarkable adaptability of *Aedes* sp. or

the favorable conditions provided by the water reserve, which facilitate the growth and development of *Aedes* sp. mosquitoes. It is worth noting that this sort of tank is commonly seen in such environments. Based on the aforementioned information, it can be inferred that the water reserve in a septic tank, which is commonly referred to as "dirty water," can serve as an alternate breeding location for *Aedes* sp. mosquitoes, resulting in the creation of a new ecosystem for these mosquitoes.

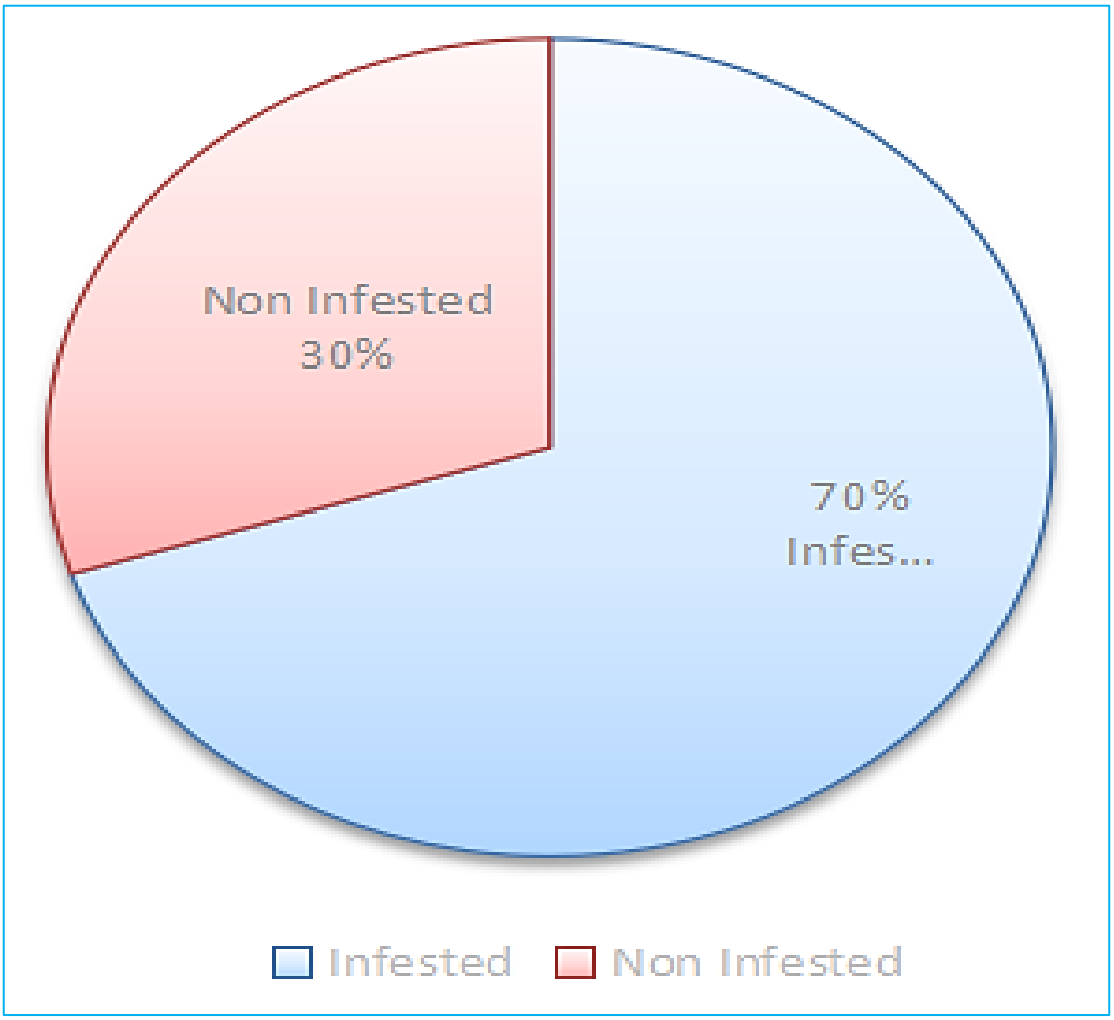

Fig 3 Graphical representation of infested (70%) and non-infested (30 %) tank

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