Original Article

Prevalence of Malaria, Dengue, and Chikungunya Significantly Associated with Mosquito Breeding Sites

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Abstract

Objectives: To observe the prevalence of malaria, dengue, and chikungunya and their association with mosquito breeding sites.

Methods: The study was observational and analytical. A total of 162 houses and 670 subjects were observed during the study period. One hundred forty-two febrile patients were eligible for the study. After obtaining informed consent from all febrile patients, 140 blood samples were collected to diagnose malaria, dengue, and chikungunya. Larval samples were collected by the standard protocol that follows. Correlation of data was performed by Pearson correlation test.

Results: Forty-seven blood samples were found positive: 33 for chikungunya, 3 for dengue, and 11 for malaria. Fifty-one out of 224 larval samples were found positive. Out of the 51 positive samples, 37 were positive for *Aedes*, 12 were positive for *Anopheles*, and two were positive for *Culex* larvae.

Interpretation and Conclusion: Mosquito-borne fevers, especially malaria, dengue, and chikungunya, have shown a significant relationship with mosquito breeding sites.

Key words: Malaria, dengue, chikungunya, larvae, mosquito breeding sites.

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Introduction

In India, immunity to malaria is unstable, allowing people of all ages to become ill during an epidemic. In Karnataka state in 2005, 83,181 cases of malaria were reported, out of them, 21,984 were cases of *Plasmodium falciparum*. There were 26 malaria-related deaths. Dengue is a flu-like viral disease characterized by fever, rash, and muscle and joint pain. It is spread by the bite of infected Aedes mosquitoes carrying DEN-1, DEN-2, DEN-3, and DEN-4 virus strains of the flaviviradae family. In Southeast Asia, the estimated number of dengue cases is reported to be 20-30 million. In India, it is endemic as well as occasionally epidemic in metropolitan cities.[1] The chikungunya virus is a rare form of alpha virus, which is spread by *Aedes* mosquitoes and characterized by fever, rash, and arthralgia. In 2006, more than 1.25 million cases were reported in India, the majority of which were from the states of Karnataka and Maharashtra.[1]

Anopheles mosquitos prefer to breed in clean

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water in and around houses. *Aedes* mosquitos prefer to breed in artificial collections of water, *Culex* mosquitoes prefer to breed in dirty water, and *Mansonia* prefers to breed in aquatic vegetations. A low literacy rate, especially in women, a lack of knowledge of the proper disposal of solid wastes, sewage and excreta, intermittent or inadequate water supply, the lack of drainage facilities, a high rate of unskilled workers and unemployment, an unhygienic lifestyle, slum and cluster dwellings, high population density, low per-capita income, and a poor knowledge regarding vector-borne diseases and mosquito breeding sites and their preventive measures are playing a pivotal role in the transmission and propagation of the vector-borne diseases in the area, *viz.* dengue, malaria, and chikungunya.

Although people believed that the occurrence of fevers and mosquito breeding sites are interrelated, scientific study has not proven this. The present study aimed to establish this correlation.

Material and Methods

Ethical clearance for the study was obtained from the Internal Ethical Committee for Biomedical Sciences of the National Institute of Unani Medicine, Bangalore, Karnataka, India.

Study Area

The study was conducted in Bangalore, the capital of Karnataka, which is the third most populated and fifth-largest metropolitan city of India. The estimated metropolitan population of Bangalore city is 6.5 million. The study area was J.J.R. Nagar, popularly called Gouripalya, near the center of Bangalore.

Tools for Conducting the Study

Prestructured questionnaires were used for collecting data. These prestructured questionnaires had three parts: A, B, and C (Appendix). Part A was based on information regarding sociodemographic profile and questions regarding the history of fever. All 670 subjects answered Part A of the questionnaire, and only those who reported fever for fewer than 21 days completed Part B, which included questions regarding malaria, dengue, chikungunya, and other fevers. Those giving such history were asked about the treatment history in the respective primary health centers (PHCs). The surveyor recorded the temperature of febrile cases by digital thermometer and reviewed the treatment record cards given to each patient by their nearest PHC. The surveyor answered questions 14-36 of Part B after taking a history and conducting a clinical examination. The causes of the fevers were identified either as malaria, dengue, chikungunya, or other fevers. Part C contained information on mosquito breeding sites in the specific locality. The surveyor completed this part after observing breeding sites in and around dwellings. These were classified as domestic, peridomestic, or external. The surveyor collected samples from these sites and recorded the larva sample number and its volume. The samples were later taken to National Institute of Malaria Research (NIMR), where the species of mosquito were identified.

Patients who were included in the study were informed of the purpose of research and investigations to be conducted upon them and were asked to complete a consent form.

Investigations: The following investigations were carried out in each patient to confirm the diagnosis.

- Serum IgM for dengue antibody by ELISA method
- Serum IgM for chikungunya antibody by ELISA method
- Thick and thin blood films for malaria parasites by stereoscopic binocular microscope in 100x oil immersion lens.

IgM (ELISA) is the only diagnostic tool in India for dengue and chikunguniya in community-based research. All patients became IgM positive within seven to ten days for chikunguniya and within seven days for dengue.

Study type: Cross-sectional

Study design: Observational, analytical

Duration of the field study: six months (September 2008 to February 2009)

Sample size: The Breteau index of the dengue is considered 13 percent as the hypothetical lower limit for transmission of dengue.[2] Sample size was estimated by applying B.K. Mahajan "Methods in Biostatistics" sampling formula for quantitative and qualitative data.[3]

Methods of collection of data: The data were collected by a house-to-house survey in the study area. Prior permission was obtained from the local governing authority. Simple randomization method was considered suitable for collection of data. Necessary

help was requested from the local voluntary organization in translating questionnaires into Kannada and other languages and also in motivating the residents to participate in the study. The area of the study in Gouripalya included 200 houses and 1,200 dwellers, consisting of migrant workers, temporary dwellers, and permanent dwellers. One hundred and sixty-two of the 200 houses were surveyed. The remaining 38 houses were found closed during the survey period. The 162 houses had 670 dwellers of all ages and of both sexes. The subjects filled out Part A of the questionnaires. The surveyor filled out the questionnaires of those who could not complete theirs due to illiteracy or age.

Methods for collection of the blood sample: Blood samples were collected in a 5-ml disposable single-use syringe, and the blood was divided into two different test tubes *viz.* heparinized Vacuette with stopper (4 ml, NH sodium heparin 13x75; 2008-09, Greiner Bio One GmbH, Bad Halter Str; Kremsmunster, Austria) and a nonheparinized tube. The heparinized tubes were used to prepare thick and thin blood films to observe the malaria parasites, and the nonheparinized test tubes were used for the detection of dengue and chikungunya IgM antibodies. Heparinized test tubes were sent to NIMR Bangalore with proper labeling. Nonheparinized tubes were sent to Public Health Institute (PHI) in Bangalore for the detection of dengue and chikungunya IgM antibodies.

Methods for examination of blood samples for malaria parasites: Dehemoglobinized thick films and methanol-fixed thin films were stained with JSB2 and JSB1 and dried properly. The slides were examined under the high power of a binocular stereoscopic microscope for the identification of malarial parasites. Results were recorded as positive for Pf, Pv, Po, or Pm for *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale*, or *Plasmodium malarae*, respectively.[4,5]

Methods for detection of dengue and chikungunya antibodies: Nonheparinized blood samples were tested using ELISA kits supplied by the National Institute of Virology, Pune, in PHI, Bangalore. The results were recorded dichotomously as positive or negative.

Methods for collection of water samples for larval detection: The samples were collected from small containers such as plastic pots, coconut shells, tires, and mud pots by emptying water into a plastic container (300 ml capacity with tight cap). When the water container or drums were so large that emptying was not possible, the samples were collected by passing a strainer to the bottom of the container. The strainer with larvae was immersed into a plastic container with water. Samples from stagnant drains were collected with a plastic dipper (100-ml capacity). Samples were collected from cement tanks and other big reservoirs by passing a fine strainer with small pore base meshes into the water and then putting the sample in the plastic container with water. A torch was used in the dark, when the floor of the containers or reservoirs was not clearly visible. After that, the larval samples were sent to the NIMR in Bangalore with proper labeling (e.g. house number, types of container/reservoirs, date of collection, larvae sample number). Larvae were allowed to emerge into adult mosquitos for the identification of species.

Methods for assessment of larval sample: Larval samples were kept in a room at 26±2.0°C temperature and 75±5% humidity with larval feeding, consisting of a dog biscuit and yeast, and covered with mosquito cages. After two to three days, larvae became adult mosquitos after passing through pupa and molt stages. They were caught using a suction tube and anesthetized. Then the mosquitoes were put on microscopic slide for species identification.

Analysis of Data: Collected data were analyzed by Pearson-Correlation test.

Results

Out of the 670 subjects filling out Part A of the questionnaires, 142 (21.1%) had fevers of <21 days and signed the consent forms that constitute the study subjects. However, two patients did not agree to give blood samples but agreed to complete the questionnaire. Therefore, only 140 blood samples were collected. There were 38 men and 104 women. The subjects ranged from young children to adults age 89. Eleven subjects suffered from malaria, three from dengue, and 33 from chikungunya. The other 95 patients suffered from other causes of fever. Malaria was caused by *P. falciparum* in four, by *P. vivax* in six and by both in one patient. *P. malariae* and *P. ovale* were not found in any of the patients. Tables 1-8 show the distribution of fever cases according to the age, occupation, socioeconomic status, duration, and grade of fever (no patients had a temperature above

Table 1. Distribution of fever diagnoses by age.

Age in years	No. of subjects	Percentage	Malaria	Dengue	Chikungunya	Other Fevers
0-14	5	3.5	0 (0%)	3 (60%)	1 (20%)	1 (20%)
15-29	37	26.1	1 (2.7%)	0 (0%)	10 (27%)	26 (70.3%)
30-44	51	35.9	6 (11.8%)	0 (0%)	8 (72.5%)	37 (72.5%)
45-59	32	22.5	3 (9.4%)	0 (0%)	7 (21.9%)	22 (68.8%)
60-74	13	9.1	0 (0%)	0 (0%)	7 (53.8%)	6 (46.2%)
75-89	4	2.8	1 (25%)	0 (0%)	0 (0%)	3 (75%)
Total	142	100%	11 (7.7%)	3 (2.1%)	33 (23.2%)	95 (66.9%)

Table 2. Distribution of study subjects according to occupation.

Occupation	No. of Patients	Malaria	Dengue	Chikungunya	Other Fevers
Managerial	5 (3.5%)	0(0%)	0 (0%)	2 (40%)	3 (60%)
Skilled	14 (9.9%)	4 (28.6%)	0 (0%)	4 (28.6%)	6 (42.9%)
Unskilled	26 (18.3%)	0 (0%)	1 (3.9%)	5 (19.2%)	20 (76.9%)
Homemaker	71 (50%)	7 (9.9%)	0 (0%)	14 (19.7%)	50 (70.4%)
Other	26 (18.3%)	0 (0%)	2 (7.7%)	8 (30.8%)	16 (61.5%)
Total	142	11 (7.8%)	3 (2.1%)	33 (23.2%)	95 (66.9%)

Table 3. Distribution of subjects by socioeconomic status.

Monthly Income (thousands of INR)*	No.	Percent	Malaria	Dengue	Chikungunya	Other Fevers
>10	21	14.8	0 (0%)	0 (0%)	7 (33.3%)	14 (66.6%)
5-10	26	18.3	4 (15.4%)	1 (3.9%)	11 (42.3%)	10 (38.4%)
<5	95	66.9	7 (7.4%)	2 (2.1%)	15 (15.8%)	71 (74.7%)
Total	142	100%	11 (7.8%)	3 (2.1%)	33 (23.2%)	95 (66.9%)

* 1 USD = 48 INR approximately

39.0°C or 104.0°F), types of fever, signs and symptoms, and joint pain. Tables 9 and 10 show the distribution of patients by sex and education.

Two hundred twenty-four water samples were collected for larvae study. Of these, 185 samples were collected from domestic sites, and 31 (16.8%) were found positive for larvae. Thirty-three samples were collected from peridomestic sites, and 14 (42.4%) were found positive for larvae. All the six samples from external sites were positive for larvae (Table 11). The number of positive larvae samples were significantly correlated with domestic and peridomestic breeding sites (r=0.999, p=0.0015, and r=0.996, p=0.0036, respectively, but not with external breeding sites (Table 12). A total of 51 samples were positive for larvae. Out of these, 37 were positive for *Aedes*, 12 were positive for *Anopheles*, and two were positive for *Culex.* Table 13 shows the relationship between the fever diagnosis and larvae type (r=0.999, p=<0.0001). *Culex* mosquitoes only transmit lymphat-

Table 4a. Distribution of patients according to duration of fever

Days	Number	Percent
0-9	91	64.1
10-15	41	28.9
16-21	10	7.0
Total	142	100%

Table 4b: Distribution of patients according to grade of fever

Grade Temp(ºF)	Number	Percent
Mild (99-99.9)	5	3.5
Moderate (100-102.9)	128	90.1
High (103-105)	9	6.3
Total	142	100%

Table 5. Distribution of grade of fever according to diagnosis

Diagnosis	Mild (99-99.9ºF)	Moderate (100-102.9ºF)	High (103-105ºF)	Total
Malaria	0 (0%)*	9 (6.3%)	2 (1.4%)	11
Dengue	0 (0%)	2 (1.4%)	1 (0.7%)	3
Chikungunya	0 (0%)	28 (19.7%)	5 (3.5%)	33
Others	5 (3.5%)	89 (62.7%)	1 (0.7%)	95
Total	5	128	9	142

*Percentage of total subjects, n=142

Table 6. Distribution of patients according to types of fever

Types of Fever	No.	Percentage	Malaria	Dengue	Chikungunya	Other
Continuous	50	35.2	0 (0%)	1 (2%)	1 (2%)	48 (96%)
Remittent	69	48.6	0 (0%)	2 (2.9%)	29 (42.0%)	38 (55.1%)
Intermittent	23	16.2	11 (47.8%)	0 (0%)	3 (13.0%)	9 (39.1%)
Total	142	100%	11	3	33	95

ic filariasis in India, which was not included in our study.

Discussion

The present study was conducted to find out the relationship between fevers and mosquito breeding sites and thereby vector density. The study statistically proved the hypothesis of Greco-Roman and Arab physicians that fever mostly occurs in marshy, wet, humid, and rainfall dominant areas or swamps.

One study conducted in Calcutta, India, 10 years ago, revealed that 4.4 percent of 379 blood samples were positive for chikungunya. However, in our study 33 (23.6%) sero-samples were found positive for chikungunya. The difference between the two studies may be due to repeated epidemic outbreaks of chikungunya fever in Karnataka during the last few years.

In our study, 22.8 percent of larvae samples were positive for larvae of *Anopheles*, *Aedes*, and *Culex* species. One study conducted in rural area of Bangalore by N. Issacs[2] reported that 6.7 percent of larvae samples were positive for *Aedes*, but our study showed that 16.5 percent of larval samples were positive for *Aedes*. It may be due to poor environmental sanitation in the studied urban area. A total of 224 larval samples were collected for the study, and out of them 51 samples were positive for larvae; therefore, the container index of the study area was 22.8 percent. Out of 51, 37 were positive for Aedes, 12

Table 7. Distribution of patients by signs and symptoms

Signs and Symptoms	Number	Malaria		Dengue	Chikungunya
		Pf+	Pv+/Pmix		
Fever with chills and rigor	81 (57.0)*	4	7	1	17
Fever with typical paroxysm**	12 (8.5)	4	1	0	1
Fever with rashes	1 (0.7)	0	0	1	0
Fever with impaired consciousness	5 (3.5)	4	1	0	0
Palpable liver	3 (2.1)	2	1	0	0
Palpable spleen	9 (6.3)	2	1	0	0
Convulsion	2 (1.4)	1	1	0	0
Anemia	65 (45.8)	3	2	3	17
Fever with vomiting	20 (14.1)	4	3	1	5
Dehydration	100 (70.4)	4	4	3	22
Blurred vision	2 (1.4)	1	1	0	0

*percentage of total subjects, n=142
**cold, hot, sweating, relative bradycardia, hypotension, dehydration
Pf+: *Plasmodium falciparum*, Pv+: *Plasmodium vivax*, P mix (mixed/combined occurrence): Pf+Pv

Table 8. Distribution of patients according to joint pain

Joint pain	Number	Percentage	Malaria	Dengue	Chikungunya	Other
Mild	44	30.9	0	0	11	33
Moderate	11	7.8	0	0	11	0
Severe	13	9.2	1	0	11	1
None	74	52.1	10	3	0	61
Total	142	100	11	3	33	95

Table 9. Distribution of patients according to sex

Sex	Number	Percentage	Malaria	Dengue	Chikungunya	Other
Male	38	26.8	3 (7. 9%)	0 (0%)	10 (26.3%)	25 (65. 8%)
Female	104	73.2	8 (7.7%)	3 (2.9%)	23 (22.1%)	70 (67.3%)
Total	142	100	11	3	33	95

were positive for Anopheles, and two were positive for Culex. Out of 37 Aedes larvae, 27 were *Aedes aegypti,* six were *Aedes vittatus*, and four were *Aedes albopictus*. All 12 of the *Anopheles* were female *Anopheles stephensi*. The two *Culex* larvae were *Culex quinquefasciatus*. We conclude that the *Aedes aegypti* was the main vector for transmission of dengue and chikungunya, *Anopholes stephensi* is the urban vector for transmission of malaria, which is in agreement with previous studies.[6-9] *Culex* is the vector for lymphatic filariasis in India, which was not observed in our study samples.

In conclusion, this study proved a definite relationship between studied vector-borne fevers and

Table 10. Distribution of patients according to educational qualification and diagnosis

Class	Number	Percentage	Malaria	Dengue	Chikungunya	Other
Illiterate	33	23.2	3 (9.1%)	2 (6.1%)	7 (21.2%)	21 (63.6%)
Primary	5	3.5	0 (0%)	0 (0%)	1 (20.0%)	4 (80.0%)
Middle	32	22.5	3 (9.4%)	1 (3.1%)	11 (34.4%)	17 (53.1%)
SSLC	50	35.2	5 (10.0%)	0 (0%)	7 (14.0%)	38 (76.0%)
PUC	15	10.6	0 (0%)	0 (0%)	5 (33.3%)	10 (66.7%)
UG/PG	7	4.9	0 (0%)	0 (0%)	2 (28.6%)	5 (71.4%)
Total	142	100	11 (7.8%)	3 (2.1%)	33 (23.2%)	95 (66.9%)

Primary (1-5), Middle (6-8), SSLC: Senior Secondary Level College (9-10), PUC: Pre-University College (11-12), UG: Undergraduate, PG: Postgraduate

Table 11. Distribution of breeding sites (in and around)

Type of Breeding Site	Number Observed	Number Positive for Larvae	Percentage
Domestic	185	31	16.8%
Peridomestic	33	14	42.4%
External	6	6	100%
Total	224	51	22.76%

Domestic = metal container, earth pot, steel container, plastic drum; peridomestic = tank and cement tank tank
External = broken glass, drain and tire

Table 12. Relation of larvae positivity to type of breeding site

Type of Larvae	Domestic	Peridomestic	External	Number Positive Samples
Aedes	23	10	4	37
Anopheles	8	4	0	12
Culex	0	0	2	2
Total	31	14	6	51

Table 13. Relation between fever diagnosis and larvae type

	Diagnosis	Positive larvae
Aedes-related fevers	36 (Dengue-03, Chikungunya-33)	37 (*Aedes*)
Anopheles-related fever	11 (Pf-04, Pv-07)	12 (*Anopheles*)
Culex-related fever	0 (Lymphatic Filariasis) *	2 (*Culex*)
Total	47	51

*No case of lymphatic filariasis was found in Bangalore, vector-borne disease surveillance report, 2008.
r=0.9988, CI=0.9792 to 0.9999, p 0.0001 (Pearson correlation)

various types of vector breeding sites. We can break the chain of transmission of infection by eliminating breeding sites in and around dwellings, which simply requires commitment.

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Appendix

Prestructured Questionnaires

Part A

1. Name of the patient:
2. Father's/Husband/Guardian's name:
3. Contact Address:

House Number: Road Number: Contact Number:

4. Family members (Total):
5. Age and Sex:
   a) Child (3 months to 14 years) i) Male ii) Female
   b) Adult (More than 14 years) i) Male ii) Female
6. Religion: a) Hindu b) Muslim c) Christian d) Other
7. Marital Status: a) Married b) Unmarried
8. Occupation: a) Managerial b) Skilled c) Unskilled d) Homemaker e) Other
9. Educational qualification: a) Ignore b) Literate c) Ten class d) Twelve class e) Graduate f) PG
10. Income (Monthly):
11. Immunity status: a) Vaccinated b) Nonvaccinated
12. Addiction (if any): a) Alcohol b) Drugs c) Tobacco, tobacco product use
13. History of fever: a) 0-9 days b) 10-21 days c) More than 21 days

Prestructured Questionnaires

Part B

14. History of known fever: a) Chikungunya b) Dengue c) Malaria d) Other
15. History of fever in any family member within the previous month:
    a) Chikungunya b) Dengue c) Malaria d) Other
16. History of travelling:
    a) Time
        i) Short term(< one month) ii) Long term (≥ one month)
    b) Place:
    c) Staying condition:
        i. Tribal (Forest related areas) ii. Rural (Irrigation area)
        iii. Urban and periurban iv. Project area
17. H/O medicine/antimalarial drugs/antimicrobial medicine within one month (if any):
18. History of hospitalization within one month:
    a) Yes b) No
19. History of blood transfusion:
    a) Yes b) No
20. History of any surgery or others:
    a) Yes b) No
21. Recorded temperature:
    a) Normal (36.9C/98.4F) b) Pyrexia (38.5C/102.0F)
    c) Hyperpyrexia (39.0C to 42.0C/104.0F to 112.0F)
22. Fever: a)Fever with localizing signs
    b) With out localizing signs
23. a) Types of fever:
        i. Continuous ii. Remittent
        iii. Intermittent iv. Hectic
    b) Fever every second day (Tertian):
        i. Yes ii. No
    c) Fever every third day (Quartan):
        i. Yes ii. No
    d) Fever with rash:
        i. Yes ii. No
    e) If yes, what is type of rash:
        i. Morbiliform rashes blanches under pressure
        ii. Macular iii. Papular iii. Vesicular
    f) Pattern of rashes:
        i. Centrifugal ii. Centripetal
        iii. Unilateral iv. Bilateral v. Others
    g) Fever with hemorrhage:
        i. Yes ii. No
    h) Fever with chills and rigor:
        i. Yes ii. No
    i) If present:
        i.Typical paroxysm (cold, hot, sweating, relative bradycardia, hypotension, dehydration)
        ii. Atypical paroxysm

j) Fever with impaired consciousness:
i. Yes ii. No

24. Vomiting: a) Yes b) No
25. Headache: a) Yes (retro-orbital/occipital/general) b) No
26. Joint pain: a) Present (mild/moderate/severe) b) Absent
27. Anemia: a) Present b) Absent
28. Dehydration: a) Present b) Absent
29. Liver: a) Palpable b) Not palpable
30. Spleen: a) Palpable b) Not palpable
31. Dyspnea: a) Present b) Absent
32. Lung: a) Normal vesicular sound b) Abnormal VBS
33. Eye: a) Vision:
i. Normal ii. Blurred vision
b) Color:
i. Normal
34. Convulsion: a) Present b) Absent
35. Coma: a) Present b) Absent
36. Investigations:
a) Slide code No b) Slide examination report (For malaria)
c) Dengue and or chikungunya antibodies (IgM/IgG):
i. Positive ii. Negative

Prestructured Questionnaires

Mosquito Breeding Sites - Part C

1. Peridomestic: a) Cement tank b) Broken glass
c) Tires d) Other
2. Domestic: a) Tanks b) Earthen and plastic drum
c) Plastic bucket d) Metallic container e) Other
3. External: a) Tanks b) Tree hole
c) Coconut shell d) Others
4. Larva containing sample No.:
5. Larva sample volume:
6. Sample examination report:
a) Larva b) Pupa