Health Risk Factors for Incidence of Dengue Virus Infection in Different Districts of Punjab, Pakistan

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Background:

Dengue fever is one of the major threats all over the world. A positive-stranded RNA virus that belongs to the family Flaviviride, known as DENV is the etiological agent of dengue fever among humans. It is a mosquito-borne viral disease in humans which is spread by female *Aedes* mosquito. Female mosquito feeds on blood to mature their eggs and during their meal, they transmit the virus from infected to healthy individuals by biting. This disease is now an emerging threat in Pakistan.

Objectives:

The present study aimed to explore health risk factors for dengue fever in addition to their demographic status (age, gender, occupation, and educational level), daily work activity (sedentary or non-sedentary), and residential status (nature of house, location of house).

Material and Methodology:

The data was collected from 31 districts of Punjab Pakistan. Demographic data of all the patients was collected in a questionnaire-based Performa. The age group of different patients and the prevalence of dengue fever were correlated with each other at $P < 0.05$ by the Two-Way ANOVA Test (α significance at 0.05).

Results:

The data was collected from 31 districts of Punjab in questionnaire-based Performa. The study was conducted under ERC No 892 by the Ethics Review Committee of the Department of Biology, PMAS Arid Agriculture University Rawalpindi, Pakistan. Out of 423 patients present in District Headquarters (DHQs) hospitals in different districts of Punjab, 263 were females which compose 62% of the total affected population and 160 were males which composed 38% males were found to be the total affected population. Most of the affected females were aged between 39-47 years however most of the males were from age groups of 30-38 years.

Conclusion:

Obtained data revealed that low socioeconomic status and crowded houses with poor ventilation systems make people prone to infection. Moreover, uncovered water containers and animal/plant pots were recorded as significant indoor breeding sites for mosquitoes. However, water reservoirs and vegetation around the house were significant outdoor breeding sites.

Keywords: Dengue Fever, Human Health, Hazards, Punjab, Pakistan

Introduction: In now a day's one of the most significant arthropod-borne infections is Dengue fever (DF). Principal vectors for the transmission of dengue infection are Aedes aegypti and Aedes albopictus. Aedes aegypti is the main arthropod that is responsible for the transmission

of dengue viruses among hosts. However, Aedes albopictus comes second but is less effective for transmission of dengue disease, which feeds on various species of vertebrates [1].

Dengue fever is caused by the dengue virus (DENV) which is a positive-strand RNA virus and belongs to the flavivirus genus of the family Flaviviridae [2]. The four serotypes of DENV have been reported to be circulating all over the world. These Four serotypes of DENV termed DENV-1, DENV-2, DENV-3, and DENV-4 are considered to be etiological agents for dengue disease [3].

Dengue has been present for centuries. In 922 AD first signs and symptoms compatible with dengue were noted in the Chinese medical encyclopedia [4]. Out of four DENV serotypes, three have been co-circulating in Pakistan. In 2008, Lahore got 1800 positive cases with three dengue serotypes (DEN 2, 3 & 4) and a high frequency of DHF. In the year 2009, serotypes DEN 2 and DEN 3 were predominant [5].

If a female *Aedes aegyptii* bites during the viremic phase of an infected person then it can transmit infection. Once the infected blood is ingested the virus starts to replicate within epithelial cells of the midgut after which it enters in haemocoel from there it has access to salivary glands and saliva causing disease during bite. The virus also infects the genital tract by entering developed eggs at the time of egg laying. It took 8-12 days for the extrinsic incubation period (EIP) to last but the mosquito remains infected for its whole life [6].

The life cycle of *Aedes* is divided into two stages, namely terrestrial and aquatic. The terrestrial stage includes adult mosquitoes and eggs, whereas immature stages of larvae and pupae compose the aquatic stage. This life cycle takes 1-2 weeks or longer to complete but this period depends mainly on temperature and availability of water and nutrients during the aquatic stage [7]. *Aedes* mosquito emerge from eggs to adults in shorter periods at elevated temperatures thus higher temperature also provides a shorter incubation period for dengue virus [8]. The following study suggests that there is a combined role of plasmablast, platelets, and complement systems for the onset of severe disease in DENV infection. The activation of complement and depletion of platelets is coincident with a higher concentration of virus-specific plasmablast in a critical phase of infection [9].

Most DENV infections either do not produce any symptoms or they may produce mild illness. However severe disease is characterized by vasculopathy and plasma leakage from vessels that may result in shock and death. It is considered that multiple factors are collectively responsible for dengue-associated vasculopathy [10].

Risk factors for dengue fever in addition to their demographic status (age, gender, occupation, and educational level), daily work activity (sedentary or non-sedentary), and residential status (nature of house, location of house).

Material and Methods:

Study Area:

A lot of work has been done on dengue vaccines but still vaccines are under clinical trials. However, due to a lack of vaccines and immunization controls, the only way to prevent dengue is to prevent mosquito bites. For this purpose, it is essential to find out the predisposing factors of dengue in human communities. In this regard present study was designed to collect data from DENV-positive patients attending government district headquarters (DHQs) hospitals. The data was collected from 31 districts of Punjab Pakistan.

Collection of Demographic Data:

Demographic data of all the patients was collected in a designed questionnaire-based performance. Performa was used to gather information about demographics and self-reported risk behaviors. To collect the data patient's information regarding their demographic status (age, gender, occupation, and educational level), daily work activity (sedentary or non-sedentary), and residential status (nature of house, location of house) were taken under consideration. The details of the included features were as follows.

Demographic Characteristics:

To analyze the patient's socio-economic status features like age, gender, occupation, residence status, and education level were included in the questionnaire.

Medical History of The Patient:

The network of disease spread and understanding the possible modes of acquiring disease was a vital piece of information for the eradication of the disease. During interviews, patients were asked whether one had a blood transfusion as it indicated nosocomial infections. Patients were also asked about their history of having dengue in the past few months. The study was conducted under ERC No / Article No 892 by the Ethics Review Committee of the Department of Biology, PMAS Arid Agriculture University Rawalpindi, Pakistan.

Outdoor Breeding Sites:

To understand and identify the outdoor breeding sites of mosquitoes, potential breeding sites were included in questionnaires. Breeding sites were divided into two groups indoor and outdoor breeding sites. For knowledge of outdoor breeding sites patients were asked about the presence of any water reservoir, brick manufacturer, disposed of cans/ tins or bottles, banana trees, public toilets, gardens, and bushes near their houses. This attribute could help us to Figure out the out-breeding sites of the mosquito population. Moreover, the conditions of houses, construction materials of houses, presence of pots for plants/ animals, room coolers as well as the number of windows in the houses provide information regarding indoor breeding sites. The number of windows in the houses depicts the ventilation system of the houses. Construction materials also provided information about the living standard of the patient as most people in rural areas live in mud houses.

Study Setting:

Department of Biology, PMAS Arid Agriculture University Rawalpindi, Pakistan

Study Duration:

One Year

Inclusion Criteria:

Male and Female patients who were mentally fit and had no psychological problems but had patients of Dengue fever in DHQ Hospitals in Punjab were taken part in this study.

Exclusion Criteria:

All those males and females who were not patients in the DHQ Hospitals in Punjab did not consent to take part in this study.

Sample Size:

Using the WHO sample size calculator, the sample size for this study was 423, based on the previous study's (50%) population parameter, 95 % confidence interval, and margin uncertainty (5 percent).

$$N = \frac{p(1-p)\,z^2}{E^2}$$

P stands for prevalence.

Z is the z-score for the selected level of confidence. The z-score represents a 95 percent confidence interval.

E stands for error margin.

The number N represents the size of the population.

Sampling Technique:

Non-probability 'Convenience sampling'

Analysis plan:

For the categorical variable, the frequency and percentage were determined using SPSS version 20. All of the information was presented in the form of tables and significance was assessed using the Two-Way ANOVA Test at P value 0.05. The Two-Way ANOVA Test was used to determine the significance between the samples as well as within the samples. The

ANOVA Test handled the huge sample size easily as compared to other statistical tests such as the T-test, chi-squares test, etc.

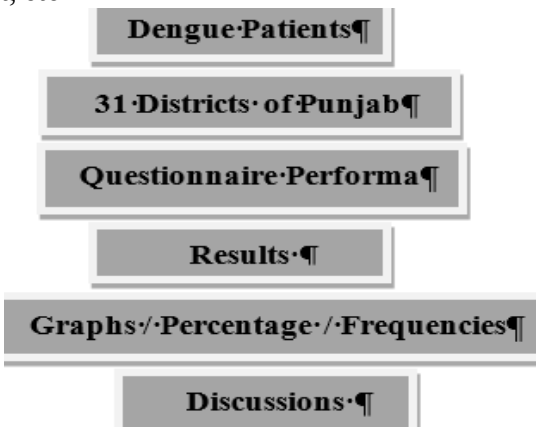

Figure 1. Flow of methodology

Results and Discussion:

Age:

In the present study, various age groups were observed to be affected by dengue virus. According to the obtained data, minimum age recorded was 12 years while the maximum age was 87 years. Patients were grouped in 9 class intervals from a minimum age of 12 up to 92 years. In the present study, only 15 (3%) persons were below age 20 while the number of cases periodically increases in age groups from 21 up to 47 as shown in Figure 1. Present results were in contradiction to a study held in 2007 which stated that the mean age of patients' affecties was 24 years in Pakistan [11].

Gender:

According to the obtained data under study, the ratio of females affected was higher than that of males. Out of 423 patients, 263 were females which compose 62% of the total affected population and 160 were males which composed 38% males were found to be affected. Most of the affected females were aged between 39-47 years however most of the males were from age groups of 30-38 years. In the present study, results are shown in Table 1 and Figure 1. The age group of different patients and the prevalence of dengue fever were related to each other at $P < 0.05$ by the Two-Way ANOVA Test (α significance at 0.05). If the P value was less than 0.05 represents that there was a strong relation between the age group and the prevalence of dengue fever in patients and if the P value is greater than 0.05 showed a weak association between the age group and the prevalence of dengue fever in patients.

Table 1. Relation between gender and age groups in different areas of Punjab (n= 423)

S. No	Age Groups	Frequency (Female)	Frequency (Male)	Two-Way ANOVA Test (P < 0.05)	Remarks Significant / non-significant
1	12- 20	15	0	0.000	Significant
2	21- 29	59	13	0.000	Significant
3	30- 38	30	77	0.000	Significant
4	39- 47	81	53	0.000	Significant
5	48- 56	30	10	0.000	Significant
6	57-65	40	2	0.000	Significant
7	66- 74	2	1	0.000	Significant
8	75- 83	3	4	0.000	Significant
9	84- 92	3	0	0.000	Significant
TOTAL		**FEMALE**		**263**	**62%**
		MALE		**160**	**38%**

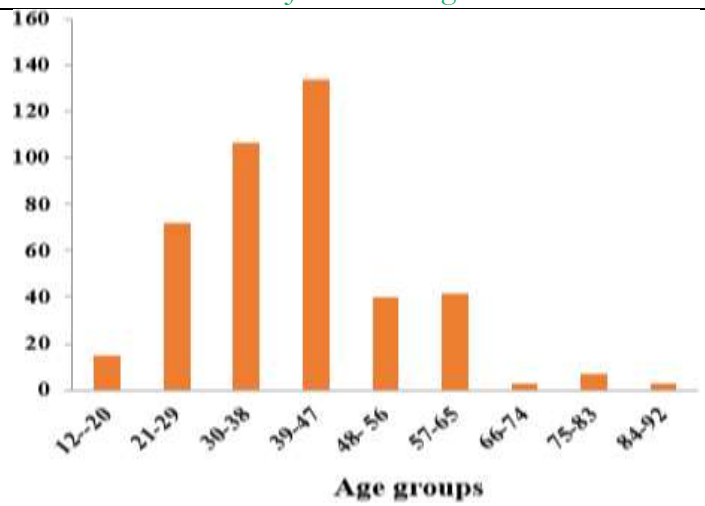

Figure 2. Frequency Distribution of Age among different gender groups in areas of Punjab (n = 423)

Occupation:

Among the studied patients, 15.83% were students while 21.04% of them were jobless or housewives. 31.9% of the total population was associated with their businesses while 31.2% was working in offices (Figure.3). However, studies reporting maximum respondents from age groups 30 and above had occupations employed or professionals as the most affected group. A study in Lahore reported 34±16.5 years as the most affected age while laborers and vocational as the most affected occupational groups [12].

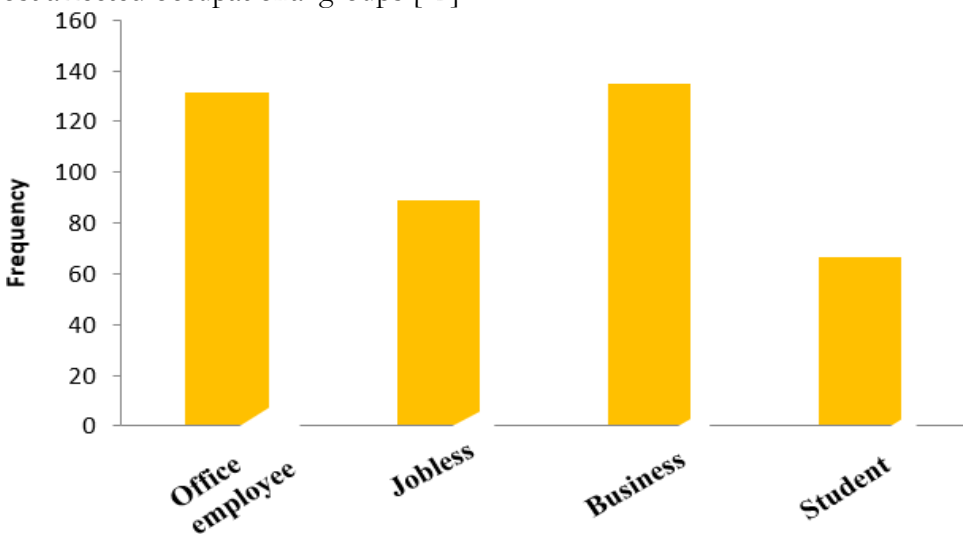

Figure 3. Frequency Distribution of Occupation in different areas of Punjab (n = 423)

Education Level of Patients:

Of the total sample, 423 patients 106 were illiterate which composes 25% of the total sample size while 29% are just primary passed. The highest peaks were recorded for the patients who have done their matriculation as 154 (36%) out of 423 patients were included in this group. It was also observed that 128 (48.66%) out of 263 females were matriculated. In addition, most of the males 49.37% were primary pass as shown in Figure 4. The urge to get better jobs forces people to travel away from their houses to seek a job which increases the risk of getting infection. While lack of higher education reduces awareness of preventive measures thus accounts for increasing risk further. Once infected these individuals spread disease to their house members and nearby areas.

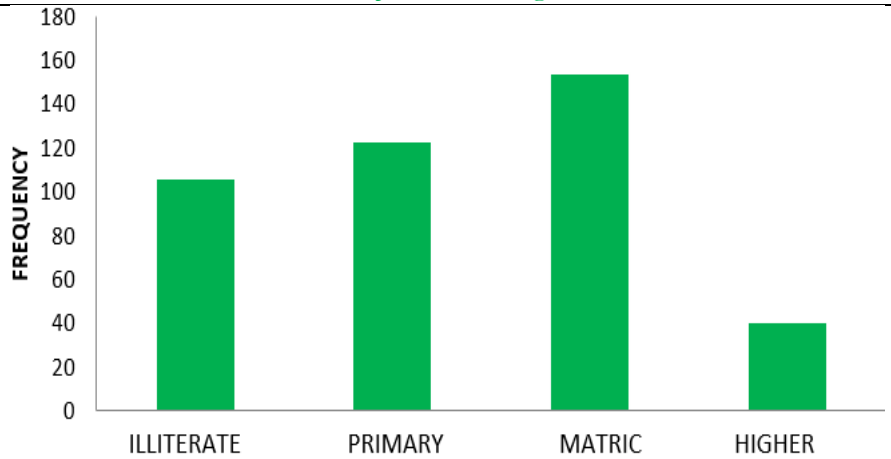

Figure 4. Frequency Distribution of Education Level in different areas of Punjab (n = 423)

Family Size and House Types:s

It was observed that most of the patients having family members 5-8 lived in open houses or houses with yards as compared to flat systems or no-yard houses. As reported 178 (42%) of individuals lived in open houses while 245 (58%) of individuals inhabited flats and had no yard. Present findings support the belief that the host density of humans plays a significant role in acquiring infection. It was revealed from the obtained data that large family sizes of 5-8 members living in open houses were more affected as shown in Figure 5. This could be attributed to the reason that in overcrowded houses susceptible individuals are close to the infected individual. The female A. agyptii can feed upon many human hosts per day thus they can effectively transmit infection [13].

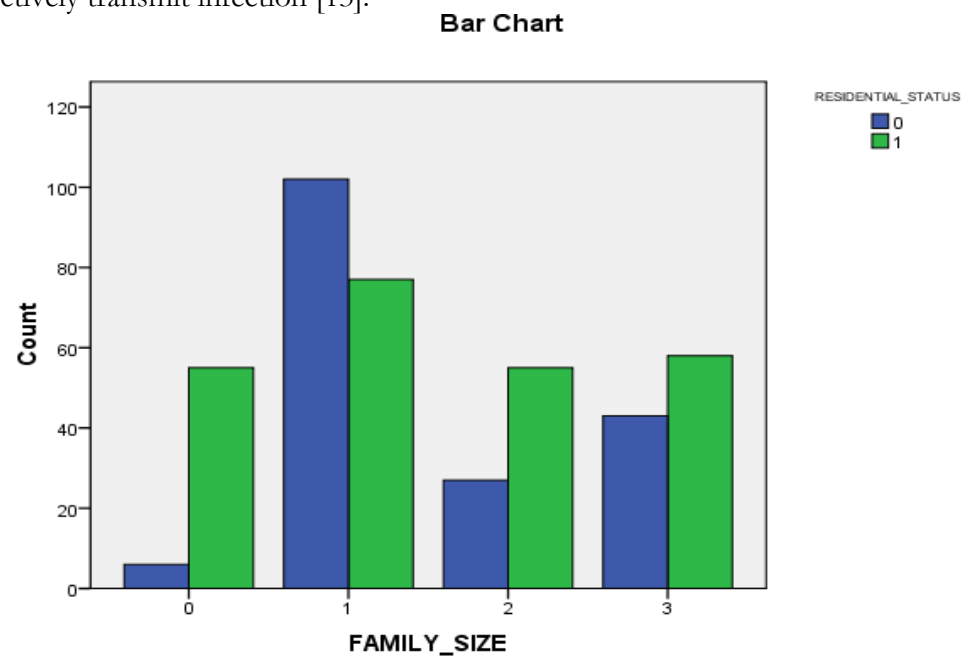

Figure 5. Comparison of family size with residential status in different areas of Punjab. (n = 423)

1-4 5-8 9-12 >12

Blue boxes show residential status '0' which is open house/ yards

Green boxes show residential status '1' which is Flats/ no yards

History of Blood Transfusion:

According to the results, 138 patients agreed that blood transfusion in the past 2 months while 285 denied a history of blood transfusion. However, due to a lack of blood samples, it

could not be concluded that there was an association between blood transfusion and the incidence of dengue.

It has been confirmed from various studies that the incidence of dengue due to blood transfusion was a rare event. Yet only five confirmed cases were reported to have dengue after blood transfusion [14].

History of Dengue Infection in Patients:

Dengue incidence and its association with history was also an important factor to describe. Results showed that most of the cases did not have any previous exposure to dengue fever. 67% of patients did not report any past dengue infection but 33% of the sample reported dengue fever before. A lot of studies have reported this phenomenon that a person who had an infection once develops antibodies against it but there were four serotypes of dengue DEN-1, DEN-2, DEN-3, and DEN-4. The antibodies developed for one serotype were incapable of neutralizing the effect of another serotype [15]. It was also reported that once infected with the virus A. aegypti can transmit this virus for its entire life [16].

House Construction Material

To determine the association of dengue incidence with house construction materials four categories of houses were made one made of wood, mud, cement, or bricks. Data revealed that the most affected were those who inhabit mud houses as shown in Figure 6. 129 (30%) reside in mud houses the second highest peak was recorded for wood houses 117 (28%) out of 423. Present results explained the phenomenon that mud houses were good regulators of temperature. The temperature inside the cemented houses was relatively lower than outside and these houses could also maintain high humidity. This atmosphere created a favorable environment for adult mosquitoes to feed and propagate.

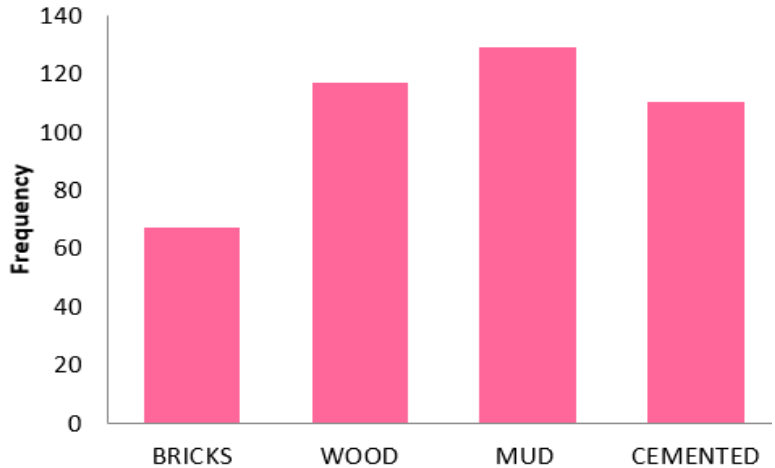

Figure 6. Frequency Distribution of House Construction Material in different areas of Punjab (n = 423)

Water Storage Containers:

Water serves as the most important factor for a mosquito to complete its life cycle. The present study revealed that 82% of the individuals used uncovered pots for their water storage at home while 18% used covered pots as shown in Figure 7. A study of east Ethiopia reported that *A. aegyptii* could flourish in a variety of artificial water storage vessels 87.6% of the presence of those vessels was uncovered or partially covered [17]. It is reported that like all mosquitoes three stages of *A. aegypti* were dependent on water [18]. Thus, the presence of water played a significant role in mosquito development and thus in dengue transmission.

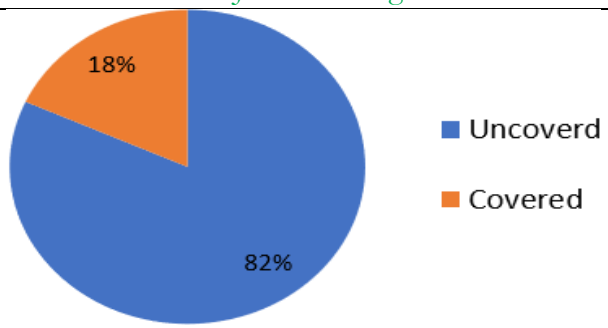

Figure 7. Percentage distribution of water storage containers in different areas of Punjab (n = 423)

Use of Pots for Plants or Animals:

Significant results were observed for keeping the plants and animal pots in houses. As shown in Figure 8, 69% of patients had animal or plant pots while 31% refused to have any. Most of the locals rear dairy animals, poultry animals, and pets. For these animals, people stored water in large containers thus increasing the risk of infection. A study reports that pans for plants and animals had an equal number of larvae from A. aegypti and A. albopictus [19].

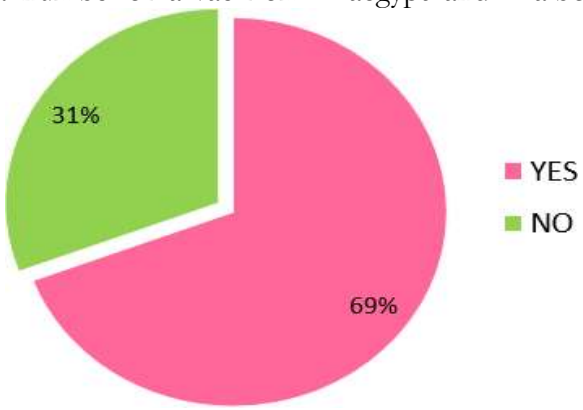

Figure 8. Percentage distribution for the presence of animals or plant pots in patients' houses in different areas of Punjab. (n = 423)

Outdoor Breeding Sites:

Presence of Garden and Bushes:

Frequency distribution of gardens shows that 71% of patients had either their own garden or had gardens in neighborhoods while 29% did not have any garden nearby as shown in Figure 9. Bushes and vegetation provided a favorable environment for mosquitoes to flourish as they provided shade against sunlight and retained humidity through transpiration. It was confirmed that most of the breeding sites of A. aegypti are under high vegetation cover [17].

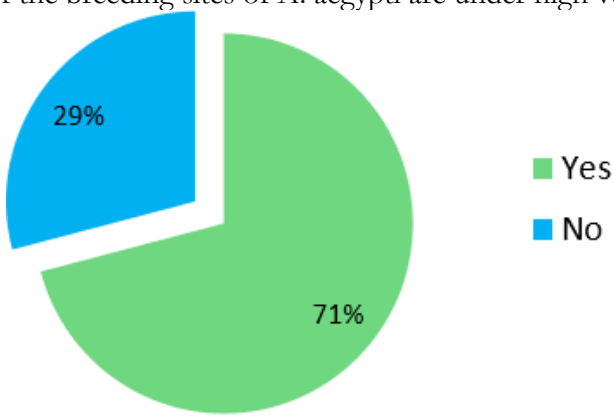

Figure 9. Percentage distribution for the presence of gardens near the house of affected persons in different areas of Punjab. (n = 423)

Presence of Tins/Cans and Brick Manufacturer:

Brick manufacturers and empty tins were sites which expected to harbor mosquito larvae. Data revealed that there was no manufacturer nearly 77% of cases and 23% evidenced that brick manufacturers were present around their houses as shown in Figure 10.

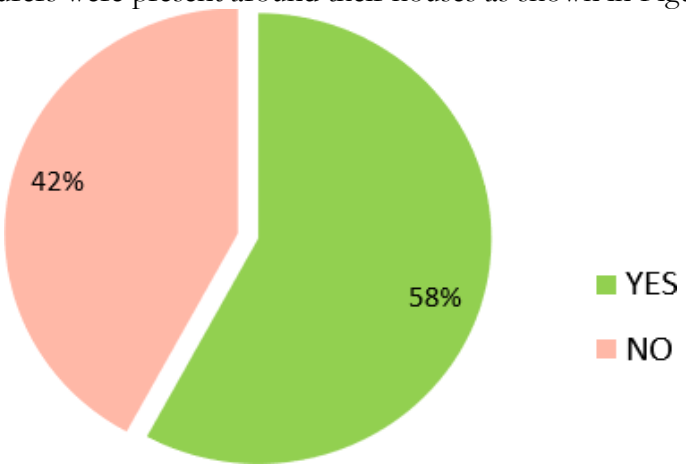

Figure 10. Percentage distribution for the presence of cans/ tins near houses in different areas of Punjab. (n = 423)

Public Toilets:

Public toilets were located near 64% of patient's residences while 36% denied their presence as shown in Figure.11. A study in Malaysia found deposited eggs of the A. aegypti in flush tanks of toilets [7]. Public toilets were usually unhygienic and dirty so there was a chance that *A. aegypti* survived there. The presence of stagnant water on the toilet floor and the lack of management regarding toilet containers provided an opportunity for mosquitoes to flourish.

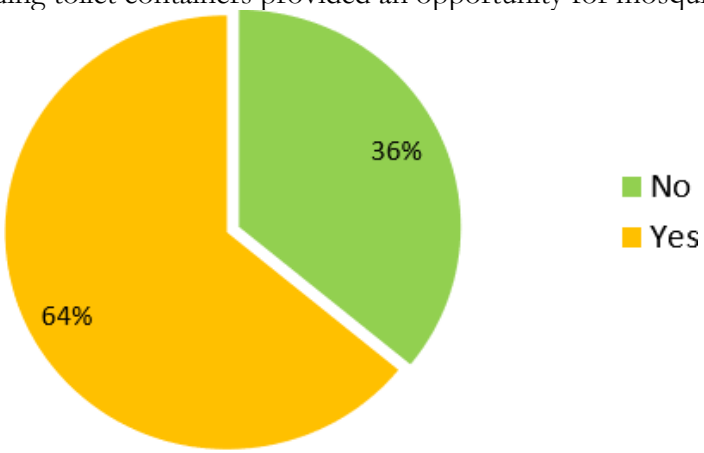

Figure 11. Percentage distribution for the presence of public toilets near houses in different areas of Punjab. (n = 423)

Water Reservoir:

Water reservoirs also had a significant effect on the incidence of dengue it was observed from data that 74% of individuals lived near or visited water reservoirs regularly. While 26% did not have easy access or did not visit to water reservoir regularly as shown in Figure 12. A present result indicated local risk factors for dengue in the province of Punjab. These findings would help dengue eradication programs to overcome the percentage of affected cases. It was suggested that biological agents for example fishes should be introduced in large water reservoirs e.g. lakes, ponds, etc fishes feed upon larvae of mosquitoes and thus reduce the incidence of dengue. However chemical agents should be sprayed on possible outdoor breeding sites as our study indicates gardens, bushes, disposed tins and public toilets were factors most reported by cases.

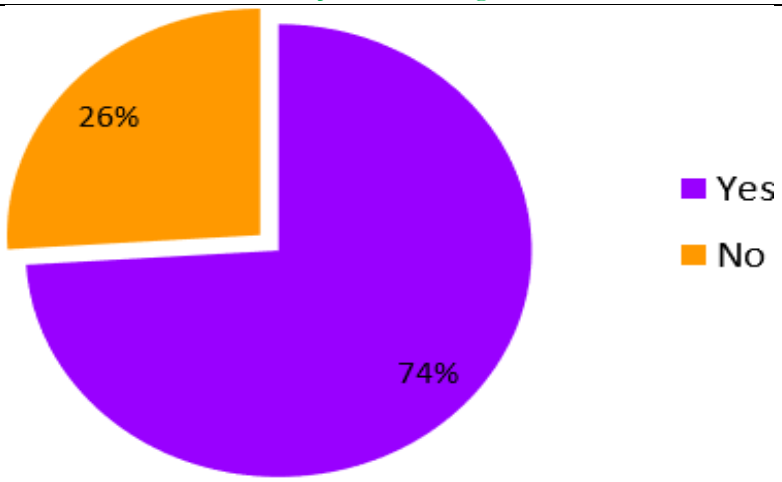

Figure 12. Percentage distribution for the presence of water reservoirs near houses in different areas of Punjab (n = 423)

The incubation period of dengue fever was from 4 to 10 days and symptoms lasted for 2-7 days. Symptoms associated with dengue were High Fever (40°C/ 104°F) (20), frontal headache, myalgia, arthralgia, and cutaneous rash. A severe form of this disease included hemorrhages, hypotension, thrombocytopenia, and plasma leakage, also accompanied by neurological alterations (21). Plasma leakage caused respiratory arrest or it led to shock along with/without serious organ impairments (20).

In terms of economic burden, in Southeast Asia on average 2.9 million dengue episodes occurred per year. These episodes made an annual cost of USD 950 million or about USD 1.55 per capita. In terms of aggregate cost Indonesia was suffering from the highest economic burden, with approx 34% of the total regional economic burden of dengue (22).

The mosquitoes were adapting to the new habitats thus contributing to the spread of dengue (23). It was reported that mosquitoes were evolving to adapt themselves to urban life (24).

Furthermore, the remarkable growth of urbanization in the past 40 years has provided suitable ecological conditions in which a large population of Aedes aegypti would flourish in close association with a dense human population thus creating the conditions ideal for dengue transmission (25).

The present study focused on factors that would increase a person's chance to contact Aedes. Results of the present study showed that as compared to children adults were more affected. This result could be attributed to the fact that most of the adults had to leave the house for work or other activities. Another reason was that most of the studies had reported the shift of age groups in countries which was the history of dengue. Other factors which showed the association of gender and socioeconomic status in the study it was observed that more affected gender were females because they were more social than men as most of the females visit their neighbors frequently more over they visit to enquire about the health of patients in the relatives and locality in addition they were in close contact water reservoirs and it was also reported that most of the affected person was from low to lower middle class.

Conclusion: Obtained data revealed that low socioeconomic status and crowded houses with poor ventilation systems make people prone to infection. Moreover, uncovered water containers and animal/plant pots were recorded as significant indoor breeding sites for mosquitoes. However, the water reservoir and vegetation around the house were significant outdoor breeding sites.

Disclaimers		
Author Contributions	Research concept and design	Maria Riaz and Sobia Kanwal
	Collection and/or assembly of data	Maria Riaz

	Data analysis and interpretation	Maria Riaz, Abdul Mateen, and Usman Ahmad
	Writing the article	Maria Riaz and Usman Ahmad
	Critical revision of the article	Muhammad Alam and Irfan Mumtaz
	Final approval of the article	Maria Riaz and Sobia Kanwal
Conflict of Interest	The authors declare that there are no conflicts of interest.	
Data Availability	Data and supplements are available on request to the corresponding author.	
Funding	N/A	
Ethical Approval	Ethics Review Committee of Department of Biology, PMAS Arid Agriculture University Rawalpindi, Pakistan.	
Trial Registration	NA	
Acknowledgments	Department of Biology/Zoology PMAS Arid Agriculture University, Rawalpindi	

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