

Dengue Outbreak 2013-2014 in District Swat of Khyber Pakhtunkhwa, Pakistan and Detection of Dengue Virus in Suspected Patients

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ABSTRACT

The study was conducted on identification of dengue virus in suspected patients of 2013-14 dengue outbreak in District Swat and to conduct the population base surveillance for determining the proportion of dengue virus infection, dengue incident and disease burden. The total number of patients observed were 8927 out of which 6074 (68.1%) were male and 2853 (31.9%) were female. The total numbers of deaths reported due to dengue infection were 36. The infection rate was much higher among the age group of 15-30 years. The characteristics symptom of dengue infection was recorded as fever (100%), vomiting (70.18%), abdominal pain (50.16%), and splenomegaly (34.16%). In majority of the patient's platelet count ranged between 100,000-150,000/mm, fever duration in most of the patient was 7-15 days. The fever duration also varied among the dengue infected individuals with 17.2% patients having continuous fever up to many days, 55.1% having intermittent and 26.6% patients have remittent fever. For the conformation of DHF (dengue haemorrhagic fever) the tourniquet test was done and by the symptoms of patients' condition petechiae was observed in 18.66% and bleeding of mucosa in 2.56%.

INTRODUCTION

Dengue virus is a spherical single stranded virus that belongs to viral family, Flaviviridae and genus Flavivirus that affects mammalian and vector cells. Four various serotypes of dengue virus are acknowledged causing the disease (DENV-1, DENV-2, DENV-3 and DENV-4) and Aedes aegypti mosquito is the basic transmitter. Infection caused by one serotype provides a lasting immunity against that particular serotype, whoever subsequent infection by a different serotype increases the risk factor of developing fatal dengue (Centres for Disease Control and Prevention (CDC), 2000). Dengue fever is a fatal disease, having the symptom of severe flue, sickness, headache, pain behind

eye, vomiting and rashes on the body, and in severe cases it causes plasma leakage, watery accumulation, fatal bleeding or organ impairment.

This fever is considered as bone breaker fever in infected person. Its symptoms vary from age to age. The effected person is suspected when high fever accompanied by severe headache, pain behind eye, vomiting and red spot appears on the body. Vomiting, swollen glands, muscle and joint pains, increase breathing rate, bleeding gums, blood release during vomit, fatigue last 2 to 3 days. 24-48 hours are critical and might be fatal for the patient. Medical care if not timely provided increase the complexity and risk.

Dengue virus is an arthropod-born viral infection around the globe, 975 million out of 2.5 billion people were at risk of dengue infection from the large and the small regions of the tropical and sub-tropical area. An exhaustive increase occurred in recent years, especially in America Western Pacific and South-Eastern Asia was observed for the last 5 years. Dengue virus infection depends on the serotype and an increase of 30 times more infection rate is observed presently in both urban and rural areas. Dengue virus is a spherical single stranded virus that belongs to viral family, Flaviviridae and genus Flavivirus that affects mammalian and vector cells. Four various serotypes of dengue virus are acknowledged causing the disease (DENV-1, DENV-2, DENV-3 and DENV-4) and *Aedes aegypti* mosquito is the basic transmitter. Infection caused by one serotype provides a lasting immunity against that particular serotype, whereas subsequent infection by a different serotype increases the risk factor of developing fatal dengue (Centres for Disease Control and Prevention (CDC), 2000). Dengue fever is a fatal disease, having the symptom of severe flu, sickness, headache, pain behind eye, vomiting and rashes on the body, and in severe cases it causes plasma leakage, watery accumulation, fatal bleeding or organ impairment.

According to the survey of World Health Organization (WHO) 50 million dengue infections are reported globally every year. Now it is declared that it is an endemic in South Asia. Dengue fever covered a very large area of Pakistan. The spread of dengue infection may be contained in the sub-tropical areas due to a fine environment. This fever is considered as bone breaker fever in infected person. Its symptoms vary from age to age. The effected person is suspected when high fever accompanied by severe headache, pain behind eye, vomiting and red spot appears on the body. Vomiting, swollen glands, muscle and joint pains, increase breathing rate, bleeding gums, blood release during vomit, fatigue last 2 to 3 days. 24-48 hours are critical and might be fatal for the patient. Medical care if not timely provided increase the complexity and risk.

MATERIALS AND METHODS

Immunochromatographic method for detection of NS1 antigen

The SD BIOLINE Dengue rapid kit was used for confirmation of dengue infection among the clinically diagnosed dengue suspected patients. This is one step assay designed to detect dengue virus NS1 antigen in human serum, plasma, or whole blood.

Procedure

With a disposable dropper 3 drops of serum were added into sample well. As the test begins to work, purple colour moves across the result window. Test results were interpreted at 15-20 min.

Interpretation

The presence of one colour line (C band) within the result window indicates negative result. Whereas presence of two colour lines (T band and C band) within the result window indicates positive result. If the colour line was not visible within the result window, then the test was considered as invalid.

Detection of Dengue infection breeding sites method and material of affected area

For identification of Dengue vector site a survey was conducted in collaboration with District Health officer Gulkada District Swat. During the Survey, dengue vector breeding sites, i.e. fresh water standing sites were identified and samples were collected in plastic bottles which were clearly labeled. For identification of breeding site 30-40 ml of water sample were collected from each site. These samples were later observed under microscope for determination of vector larvae presence.

Affected Area

For identification of affected area by dengue infection, the suspected patient’s data were recorded on a questioner containing patient information along with their locality.

Prevailing weather condition

The data regarding the prevailing weather condition i.e. average temperature during the dengue virus infection pertaining to the 2013-14 dengue outbreak were collected from Meteorological office of district Swat.

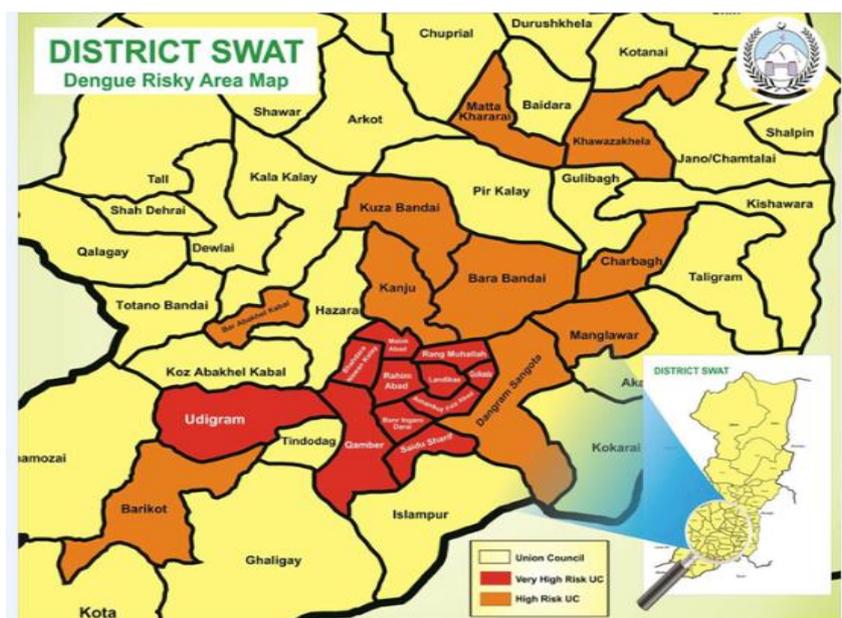
RESULTS

Different regions of district Swat were brought under considerations which were adversely effected by dengue virus including Mingora, Kanju, Saidu Sharif, Patehpu, Watkay, Barikot and Matta while some patients belong to rural areas of Matalan, Charbagh, Khawzakhela, Manglawar and Salampur of district Swat. The research was carried out with the collaboration of Saidu Teaching Hospital and Shifa Medical Centre. During our research five ml blood sample was collected from each patient in different hospitals along with a proforma containing the information of respective patients. The serum from the blood samples was isolated in Eppendorf tubes through centrifuge machine and preserved at -80 °C.

Dengue infection among population

Among the highly-infected Union Councils of District Swat the rate of infection was higher in urban areas as compared to rural. In Saidu Sharif infection was observed at 35%, Mingora city 27%, Matta 12%, Kanju 9%, Patehpur 04%, Charbagh 7% and Khawzakhela 6% (Figure 1).

Figure 1. Map of District Swat.



Clinical findings

The most common symptom among the infected patient reported was fever (100%), vomiting (70.18%), abdominal pain (50.16%), while in some patients skin rashes (Petechiae) (18.66%), gum bleeding (2.65%), and Splenomegaly (34.16%) was also observed.

During the current research among the patients identified with dengue fever and DHF, 1540 (17.2%) patients had continuous condition of fever up to many days, 4920 (55.1%) was presenting intermittent and 2470 (26.6%) patients with remittent fever (Figure 2).

Figure 2. Patients identified with dengue fever and DHF.



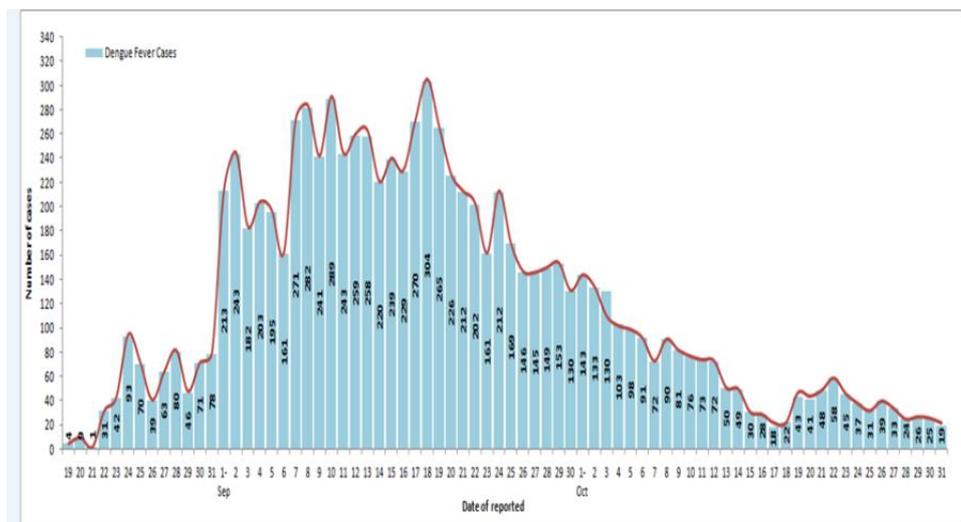
For platelets count 500 dengue patients' blood samples were tested. The platelets count in 96 (15.16%) patients were less than 50,000/cmm, in 102 (28.65%) patients it ranges from 50,000-100,000/cmm while in 302 (56.18%) patients the platelets count was from 100,000 to 150,000/cmm (Table 1).

Table 1. District Swat Tehsil Babozai (Mingora city is divided in the following high risk union councils).

District Swat Tehsil Babozai (Mingora city is divided in the following high risk union councils)	Other high risk union councils of District Swat
Rahimabad	Matta Kharirai
Banr Engaro Dherai	Khawzakhela
Gulkada	bara Bandai
Qambar	Kaza Banda
Amankot Faiz Abad	Kanju
Landi Kass	Charbagh
Odigram	Manglawar
Shahdara	Dangram Sangota
Saidu Sharif	Bar Abakhel
Rang Mhalla	Barikot
Malook Abad	

The rate of infection was low in June (14.8%) due to relatively low temperature of an average 50.2°F and gradually increased in start of the rainy season in July (17.1%) with an average temperature of 55.4°F and the rate of infection was much higher (27.1%) in August with an average temperature of 48.2°F. In September, the rate is of infection was recorded at (22.7%) due to decrease in temperature (47.3°F) and rainfall, it might be due to the well-established breeding of Aedes vectors for dengue virus) in raining months (Figure 3).

Figure 3. Dengue fever cases.



In the current research, we have calculated different outdoor breeding sites in District Swat. The data recorded is presented, The highest number of breeding sites were identified in water tanks and drums 5736 followed by tyres 4613 and plant pots 3442 while in open drain gutters the lowest number of breeding sites i.e. 72 were identified followed by water pots for tyre puncture.

We investigated 8,927 cases of Dengue fever and DHF patients out of which 6074 (i.e. 68.1%) were male and 2853 (i.e. 31.9%) females. Most of the patient belonged to the urban area and were among the age group of 15-30 years. The age and gender wise distribution ratio of dengue infected patients. Total cases of dengue infection reported= 8,927 (males=6074, females=2853). Total deaths reported to be caused by dengue infection=36 (Figures 4-10 and Tables 2, 3).

Figure 4. Cases of Dengue fever and DHF patients out of male and females.

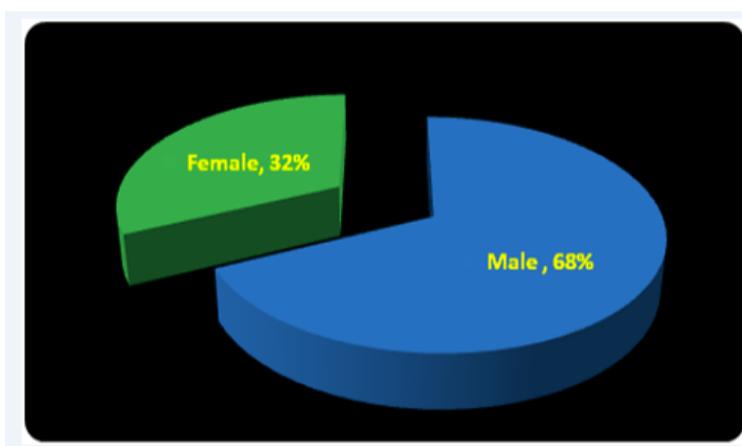


Figure 5. Age and gender wise distribution ratio of dengue infected patients.

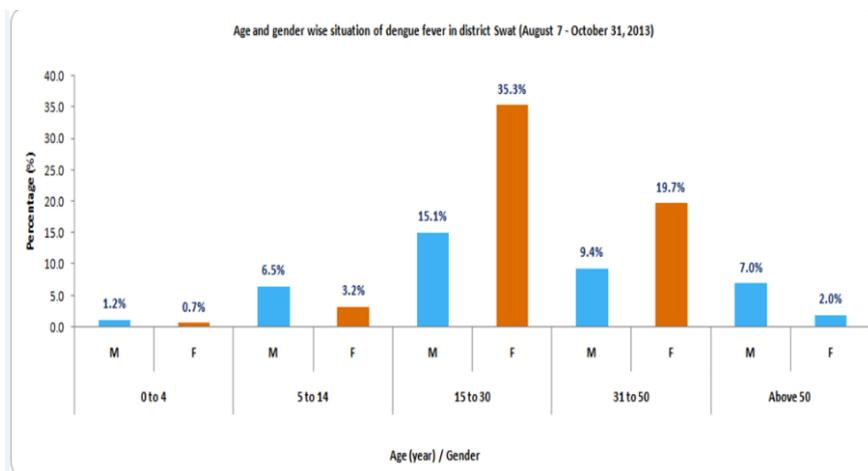


Figure 6. Percentage of cases district wise.

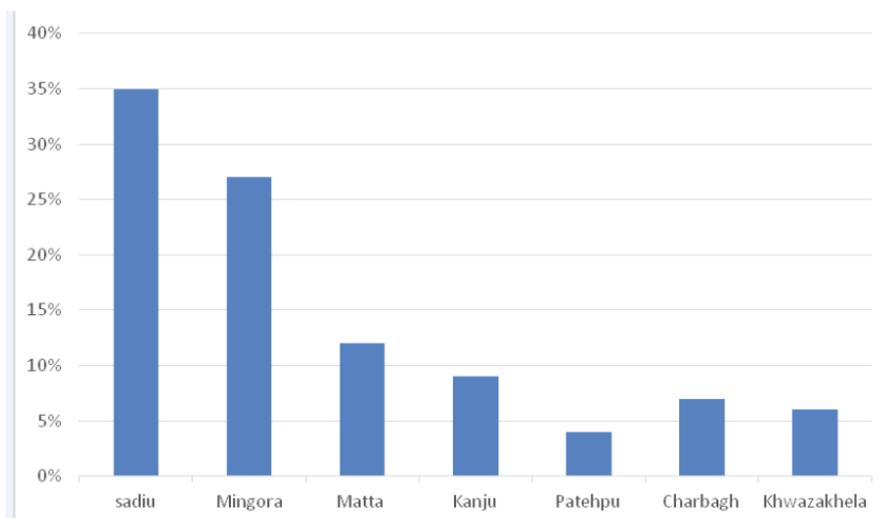


Table 2. Dengue symptoms and percentage.

Symptom	Percentage
Fever	100%
Vomiting	70.18%
Abdominal pain	50.16%
kin rashes (Petechiae)	18.66%
Gum bleeding	2.65%
Splenomegaly	34.16%

Figure 7. Dengue symptoms.

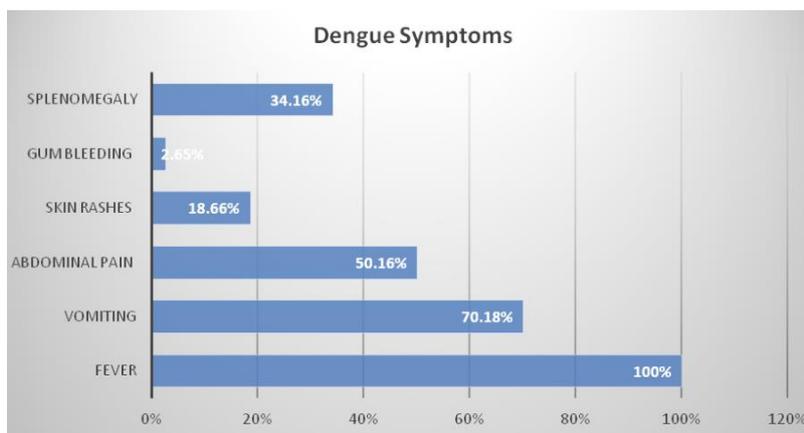


Figure 8. Durning of dengue patients.

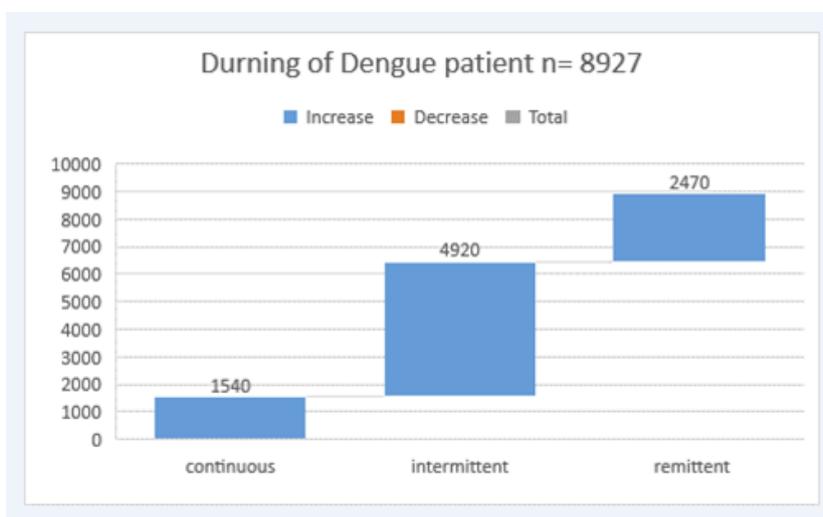


Figure 9. Monthly infection rate.

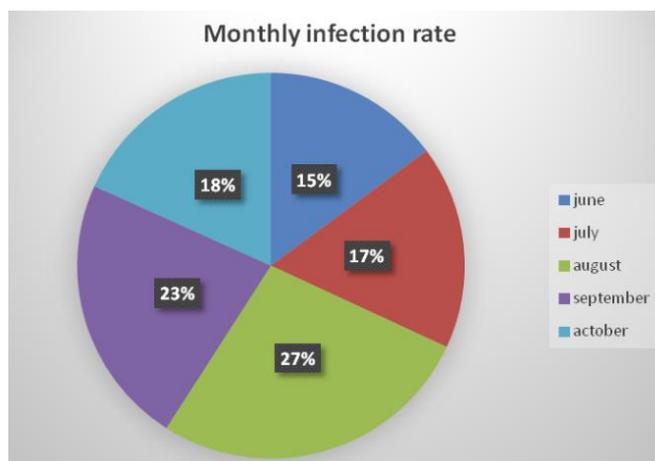
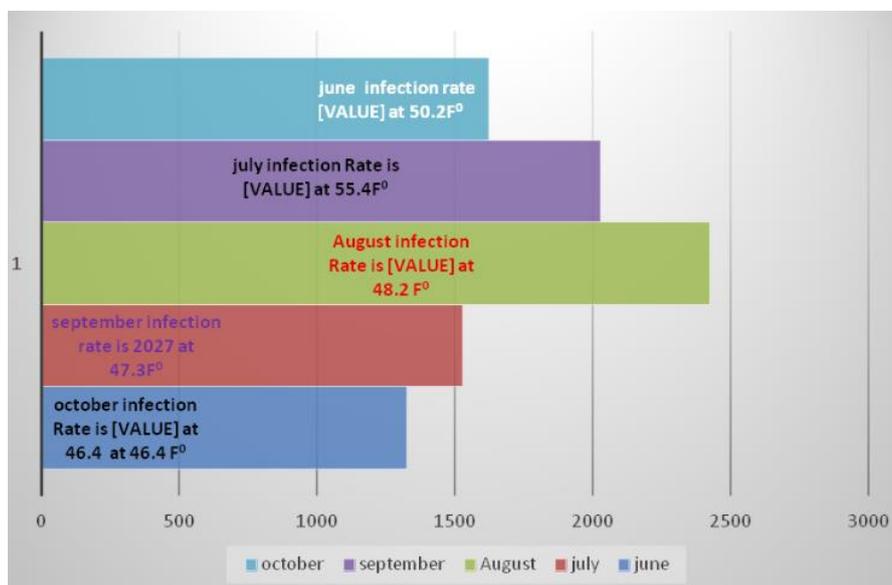


Table 3. Monthly infection rate.

June	July	August	September	October
1324 (14.8)%	1527 (17.1)%	2424 (27.1)%	2027 (22.7)%	1624 (18.2)%
50.2°F	55.4°F	48.2°F	47.3°F	46.4°F

Figure 10. Monthly infection rate.



In the current research we have calculated total number of different indoor potential breeding sites in District Swat including Standing water in the houses, leaking Water Taps, Construction waste, Cover of Gutter, Old Tyres in the houses, Mini Plant Pots, Discarded Material, Animal water utensils, Air cooler Excretion water, Store water for drinking purposes, Store water for drinking purposes, Fridge Tray, Water drums, Discarded canes on the walls, Other (discarded soil pots, plates, cold drinks bottle. The Highest Number of breeding site were identified in water Tank with 24% of the total breeding sites followed by Tyres and plant pots with 19.30 % and 14.40% respectively. While the lowest number of breeding sites were identified in water pot for tyre i.e. 1% followed by air condition water and park bush with 1 and 2% respectively (Figures 11, 12 and Table 4).

Table 4. Total number of different indoor potential breeding sites in district Swat.

S.no.	Name specific site	Number of sites	% age
1	Water tanks and drum	5736	24
2	Tyres	4613	19.3
3	Plant pots	3442	14.4
4	Standing water	2390	10
5	Waste material	1434	6
6	Other (Block drains, drums, vehicle discarded parts)	1195	5
7	Leaking water taps	1673	7
8	Bird water pots	478	2
9	Air cooler	478	2
10	Air condition water	239	1
11	Road side Hedgerow	717	3

12	Tree holes	239	1
13	Open drain/gutter	72	0.3
14	Park bushes	478	2
15	Water pot for tyre puncture	236	1
16	Construction material	478	2
	Total	23898	100

Figure 11. Total number of different indoor potential breeding sites in district Swat.

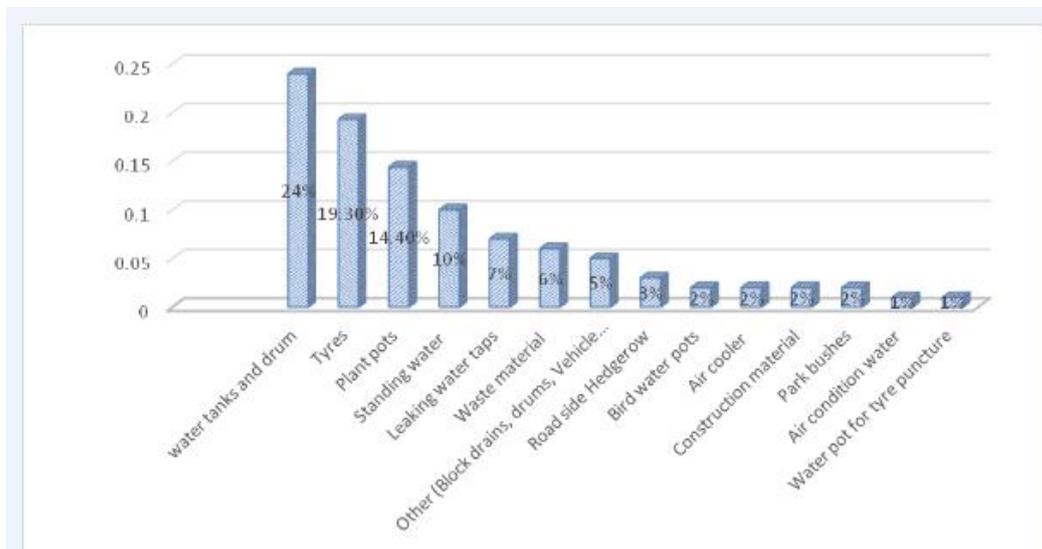
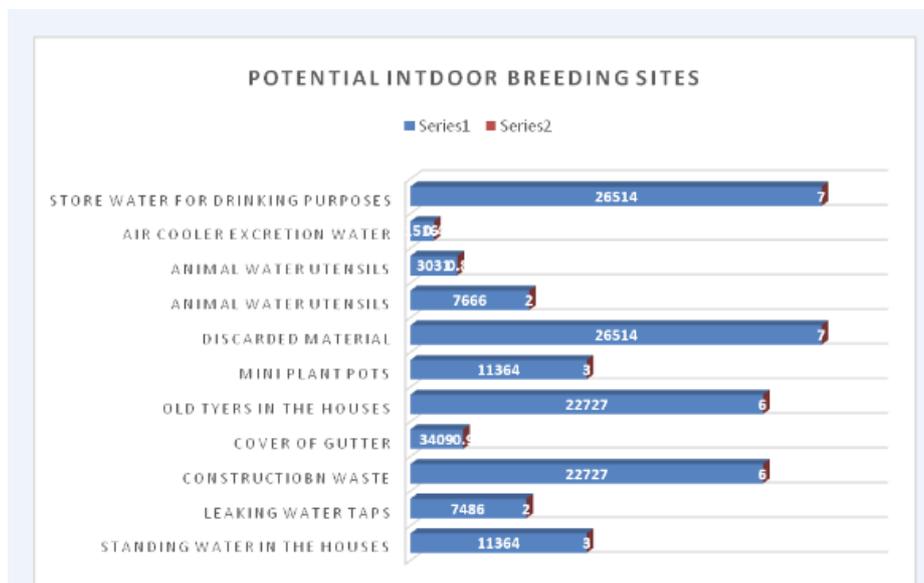


Figure 12. Potential breeding grounds.



In our current research the survey was conducted in 4 rounds for the identification of potential breeding sites and number of larva as shown. The total number of houses screened was 30,406 and the total number of breeding place were 844,076 and total number of larva recovered from different sites were 206. For the outdoor potential breeding sites, 10,685 different sites were observed and the total number of Breeding sites observed were 72,521 and the total number of larva recovered from the sites were 226 (Figure 13).

Figure 13. Potential indoor breeding sites.



DISCUSSION

Dengue has a worldwide history of about 200-400 million mostly causing infection in tropical and sub-tropical regions. Dengue virus came to Pakistan mostly through tyre imports at Karachi ports from different world destinations. Up till now the virus infection has caused outbreak in Pakistan. In 1982, dengue was identified in Pakistan. In Punjab, out of 174 suspected patients 12 were infected by dengue virus. During 1995 only two cases of dengue infection were reported in Baluchistan. DENV-1 and DENV-2 were found in patients using ELISA.

In 1985 research was conducted to identify dengue virus in Pakistan. Findings included that 20-30 % of Pakistani who lived in Karachi were haem agglutination inhibition (HI) antibody positive for West Nile, Japanese encephalitis and DENV-2 Filoviruses. The reported cases of dengue infection increase from July to October due to suitable environment. In Pakistan first outbreak was reported in 1994 in which 15 patients had dengue IgM identified using DENV-2 antigen. It was also identified that 3 out of 10 patients of dengue were infected from DENV-1 and DENV-2.

In 1995, DENV-2 virus was identified from Baluchistan province by using ELISA test. Our current research shows the circulation of dengue virus among the population of District Swat. In District Swat 2013 dengue outbreak was the first dengue infection reported in Khyber Pakhtunkhwa in which 798 dengue infections were recorded, belonging to different area of district Swat. The infection rate was found high in urban areas as compared to rural. Out of the total reported cases of dengue infection 35% infections were observed in Saidu Sharif, 27% in Mingora city, 12% in Matta, 9% in Kanji, 4% in Fatehpur, 7% in Charbagh and 6% in Khawzakhela. In 2005 outbreak of dengue fever in Karachi, DENV-3 was reported in some patients. In serum obtained from the children in Karachi DENV-1 and DENV-2 was found using serological studies.

In our research the rate of infection was low in June (14.8%) due to relatively low temperature of an average 50.2°F and gradually increased in start of the rainy season in July (17.1%) with an average temperature of 55.4°F and the rate of infection was much higher (27.1%) in August with an average temperature of 48.2°F. In September, the rate of infection was recorded at (22.7%) due to decrease in temperature (47.3°F) and rainfall, it might be due to the well-established breeding of Aedes vectors for dengue virus) in raining months DENV-2 and 3 were found to be co-circulated during 2006 in the outbreak of Karachi. In 2013 dengue outbreak in Swat out of 8927 dengue patients (male=6074 and female=2853), the rate of infection in males was high as compared to the females while

a lower infection rate was observed in both males and females below the age of 20. The case confirmation was done for anti-dengue antibodies in which NS1 positive cases among population for males and females were 6074 and 2853, respectively. Dengue strips were used for the screening of the suspected patients. DENV-3 in 2006 outbreak in Pakistan was found to be closely related to DENV-3 in 2004 outbreak in New Delhi. In 2008, a dengue outbreak was reported in Lahore infecting large number of citizens. Sample was identified to have DENV-4, DENV-2 and DENV-3 infection. In 2009, it was identified that the children live in Karachi have high level of anti-dengue IgM antibody. Some patients had co-infection with the genotype of DENV-3 and DENV-2. It showed that DENV-2 is most prevalent in the sample collected from the suspected patients. Studies showed that serotype DENV-2 was dominant in samples of dengue virus infection collected during the period of three years from 2007 to 2009.

In the month of November 2010 outbreak, it was reported by a private news channel that out of 5,050 patients, 2,350 patients were from the province of Sindh, 1,885 from Punjab province and at 158 patients from Khyber Pakhtunkhwa province. The sample had an infection with DEN-2 and DEN-1.

During 2011, the dengue infection rapidly assumed the proportion of endemic, specifically in province of Punjab and particularly in Lahore where, in the month of September, more than 250 people were reported dead as per data of Punjab health department over 12000 people were infected during the month of January to September 2011. In our current research the number of case of dengue haemorrhagic fever was between the ages of 15 to 30 year, in male population the rate of infection is comparatively high as compare to female. The patient expressing characteristic symptom of Dengue fever was hospitalized in Shifa Hospital Swat. In most of Dengue patient the fever was continuous lasting for 4 to 7 days while in some cases fever was remittent and intermittent. the anti-dengue antibody (NS1, IgG, and IgM) were indented while in most cases the NS1 was high rate as of other low platelet counter was also take in to account was also consider during this study from which we had come to know that during the dengue fever and dengue haemorrhagic fever, Platelet count gradually decreased, patient with low platelets count were at more severe condition.

Dengue infection often recorded in the form of huge outbreaks. There is also seasonality of dengue with the outbreak occurring in different period of the year. This seasonality is determining by the high rate of infection of disease which influence by the characteristics of the host, the vector and the agent. In the current study diagnosis of dengue virus infection was detection of dengue specified antibody. Serological test is available for dengue fever was IgM, IgG and NS1 antibodies strips which identified within 15 minutes which also help in differentiating between primary and secondary infection of dengue. Dr. Abdul Khaliq who's is the charge of dengue unit in Swat district health department told that the number of new patients is now decreasing and the average number of new patients has dropped from 300 to 135 per day when the temperature is reduced.

CONCLUSION

From our current study, we conclude the following:

- Dengue infection rate is higher in Male patient as compared to Female.
- The infection rate was higher among the age group of 15-30 in both male and female patients.
- Platelets count decrease during Dengue infection.
- Generally continuous fever ranging from 3 to 7 day was observed in dengue patients.
- Highest numbers of outdoor breeding sites were identified in drums while indoor most breeding site was observed in pots.
- In the Month of August most infections were observed due to relatively high humidity.
- During the final phase of disease, the infected patients were in critical condition due to bleeding and respiratory failure.
- The Signs and symptoms observed in Dengue patients were fever, vomiting, abdominal pain, splenomegaly and low platelets count.
- Highest infection rate of 35% was recorded for Saidu Sharif.

As currently no treatment/cure is available for the treatment of dengue infection, on the basis of the current study we recommend that for the prevention of dengue outbreaks ,sprays at potential breeding sites both indoor and outdoor public places may be carried out to control the dengue vector breeding sites .Awareness campaigns through seminars, conference, and sign boards are needed from government and non-government organizations through which the common people may be made aware to use repellent nets mosquito quills to reduce the likelihood of dengue infection by eliminating the vector.

CONFLICTS OF INTEREST

The authors and co-authors declare that they have no conflict of interest that affects this study.

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