

# Seroepidemiological Study of Dengue Virus Infection in Parsha District of Nepal

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## ABSTRACT

Dengue fever (DF) is an emerging mosquito borne viral disease and important public health problem in low land of Terai region which is also expanding to hilly region of Nepal. The study aims to shed light on the clinical, epidemiological and serological aspects associated with dengue virus infections (DVI) and its implications in future diagnosis, management, prevention and control of the disease in Nepal. Two hundred sixty one serum samples were collected from patients suspected of dengue virus infection visiting hospitals of Parsha districts during August- November of 2023 and tested by IgM Capture Enzyme linked immunosorbent assay (Standard Diagnostic INC., Korea) and Dengue IgM/IgG Rapid immunochromatographic test kit(Panbio, Australia).The Anti-Dengue IgM positivity was found to be 18.8% and 15.3% by IgM capture Enzyme linked immunosorbent assay and Rapid immunochromatographic test respectively. Compared to ELISA, sensitivity and specificity of RDT was 73.46% and 98.1% respectively. RDT performed poor (kappa value-0.77) and should not be used as a sole diagnostic method for diagnosis of Dengue virus infection. In 49 Anti-Dengue IgM positive cases, 67.4% were male and 32.6% were females (male to female ratio=2.06:1). The highest numbers (81.6%) cases were observed in age group of 15-50 years. Student was the most commonly affected with the highest number of positive cases (32.7%). Patients with Joint pain, retro-orbital pain and Skin rash as clinical symptoms were more likely to be diagnosed as Anti-Dengue IgM positive. Hemorrhagic manifestation was seen in 12.2% of cases. The highest numbers 199(79.6%) of cases have duration of fever more than 5 days. Anti-Dengue IgM was not found to be detected significantly in cases with duration of fever of 5 days and more ( $p=0.686$ ). Knowledge of dengue was found in 65.9% of which 11.6% was found to be Anti-Dengue IgM positive. Water logging (10.7%) and Travel to endemic area (37%) were found as the more likely risk factors in Anti-Dengue IgM positive cases. Flower pot was found as the most likely breeding place with the highest number of positive cases 36.55%. Use of net 87.3% and change stored water 85.8% was the most likely used preventive measures respectively.

### Keywords

DVI, IgM Capture ELISA, RDT, Clinical Features, Risk factors, Preventive measures

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## Introduction

Dengue is a mosquito-borne viral illness caused by one of the four serotypes of the dengue virus (DENV; (DENV-1 to DENV-4) belonging to the family Flaviviridae. The virus serotypes are closely related but antigenically distinct. Dengue infections can result in a wide spectrum of disease severity ranging from an influenza-like illness (dengue fever; DF) to the life-threatening dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) (WHO, 2008).

DF is characterized by high grade fever, sometimes biphasic, with headache, retro-orbital pain, myalgia or arthralgia, nausea or vomiting, skin rashes etc. DHF is a potentially life threatening complication of dengue characterized by high fever lasting 2 to 7 days, hemorrhagic phenomena (including vascular leakage of plasma), low numbers of platelets and sometimes circulatory failure. The condition of some patients progresses to shock. This is known as DSS (WHO, 2009; WHO, 1997). The risk of severe disease is much higher in secondary (with another serotype) rather than primary dengue virus infection (Deen *et al.*, 2006).

Dengue viruses (DVs) are transmitted to humans by mosquitoes from the *Aedes* genus; *Aedes aegypti* being the most competent epidemic vector. It lives in close association with humans because of its preference to lay eggs in artificial water holding containers in the domestic environment, and to rest inside houses and feed on humans rather than other vertebrates. Blood feeding and oviposition occur mostly in the morning and in the late afternoon. If a competent mosquito vector takes a blood meal from a person during the viremic phase (2 to 12 days), virus is ingested with the blood meal and infects the mosquito. After 8 to 12 days, depending on ambient temperature, the virus, and the mosquito, the virus will disseminate and infect other tissues, including the mosquito salivary glands. When the mosquito takes a subsequent blood meal, virus is injected into the person along with the salivary fluids.

Dengue virus infection has no apparent effect on the mosquito, which is infected for life (Gubler, 1989). Dengue is a climate sensitive vector borne disease, which in recent years has become a public health concern. Dengue is transmitting in tropical and sub-tropical regions around the world, predominantly in urban and suburban areas. Domestic Dengue Virus Infection (DVI) occurs in more than 100 countries and over 2.5 billion

people live in the areas with a risk of dengue virus infection (Gibbons and Vaughn, 2002). Up to 100 million cases of DF and 500,000 cases of DHF and several thousand deaths are estimated to occur annually worldwide. The emergence and reemergence of DF and DHF is directly related to the increase in density and geographic distribution of the vectors. In 2008, for the South East Asia region whole there was about 18% increase in the number of reported cases and about 15% increase in the number of reported dengue deaths as compared to the same period in the previous year. There was substantial increase in the reported case of dengue in Thailand, Indonesia and Myanmar. The case fatality rate in Thailand is above 0.2% in Indonesia and around 1% in Myanmar.

However, there are some outbreaks away from the urban areas that have case fatalities even up to 3 to 5% in India, Indonesia and Myanmar (WHO, 2008). During the past decades, dengue virus emerged in South Asia and DF/DHF epidemics occurred in Bhutan, India, Maldives, Bangladesh and Pakistan (WHO, 2009). The global prevalence of dengue has grown dramatically in the recent decades.

The four basic methods routinely practiced by most laboratories for dengue virus diagnosis are virus isolation and characterization, detection of DV specific antibodies, detection of Dengue antigen and detection of viral nucleic acid by nucleic acid amplification technique (WHO, 2009). Virus isolation through a mosquito cell line (C6/36) from acute phase serum or plasma sample is a method of choice and remains the gold standard (WHO, 2009; Cardoso *et al.*, 1988).

Nepal is bordered by India in the eastern, western and southern belts that is one of the countries with higher risk and so is more vulnerable to worse consequences of DVI. As with other vector borne diseases, outbreak of DF is related with increasing temperature, travel and frequent movement of people which is common due to open border between Nepal and India. DF was first reported in foreign visitor in Chitwan in 2004 (Pandey *et al.*, 2004).

Nepal reported larger outbreak in 9 districts in 2006 (WHO, 2009; EDCD, 2007). The outbreak occurred in Nepal following the Indian, Pakistan and Bhutan epidemic of DF/DHF in September-October 2006 (EDCD, 2007). The occurrence of DEN-1, DEN-2, DEN-3 and DEN-4 serotypes in the territory of Nepal augment the chances for the epidemic DF/DHF to be flourished in

the country (WHO/SEARO, 2006; Takasaki *et al.*, 2008 and Pandey *et al.*, 2008).

Several studies have reported the prevalence of DF in Nepal. The seroprevalence varies with time; 10% in 2007 (Pun *et al.*, 2011), 29.3% in 2008 (Pun *et al.*, 2011), 38.17% in 2010 (Pun *et al.*, 2012). Nepal experienced major outbreaks of DF in several districts in 2010.

During the 2010 outbreaks, DF was reported from 24 districts at Sukraraj *et al.*, (2010) Tropical and Infectious Disease Hospital alone (Pun, 2011). This indicates rapid geographical expansion is occurring within the country. Proper management of disease is required to prevent the increased threat of DVI in Nepal.

In Nepal diagnosis and management of dengue and other infectious diseases is based on clinical symptoms and many cases go undiagnosed due to lack of diagnostic facility. Thus, DF/DHF has likely been misdiagnosed and illness caused by dengue virus underestimated in Nepal. Nepal has no dengue surveillance programs, and health professionals do not usually consider dengue as a differential diagnosis (Pandey *et al.*, 2008). The 2010 epidemic of dengue indicates that dengue is increasing public health problem in Nepal. Fewer studies have been carried out for sero-prevalence of the disease in Nepal, though there is high risk of infection. This study aims to shed light into the clinical, epidemiological and serological aspects associated with DVI and its implications in future diagnosis, management, prevention and control of the disease.

The main objectives of this study that includes to determine the proportion of dengue virus infection in relation to different socio-demographic status of the patients. To determine the sensitivity and specificity of RDT and IgM Capture ELISA for the diagnosis of dengue virus infection. To describe clinical features of serologically confirmed dengue cases. And also to describe the risk factors and its impacts on dengue

## **Materials and Methods**

The study was designed as a descriptive cross-sectional. The study was carried out from August 2023 to November 2023. The total number of 261 serum samples was collected from Narayani Sub Regional Hospital and Bhawani Hospital and Research Centre, Birgunj. Serum samples were collected from individuals experiencing a febrile illness clinically consistent with dengue infection, selected according to the inclusion criteria. Patients'

personal details and clinical symptoms were obtained through a questionnaire method by direct interview in 'Dengue case details and Laboratory Investigation Form. The entire test was done at Everest International Clinic and Research Center (EICRC), Kalanki, Kathmandu.

## **Case Selection criteria**

A case was included if there was high fever with clinical symptoms suggestive of dengue infection (WHO, 2009).

## **Ethical clearance**

Written consent was obtained from all the responding patients before collecting blood samples.

## **Dengue Case Details and Laboratory Investigation Form**

I agree to provide blood samples in study titled "Seroepidemiological Study of Dengue Virus Infection in Parsha District of Nepal." and agree to be part of study (A detailed questionnaire used).

## **Sample collection, storage and transport**

The blood samples from suspected cases were collected, stored and transported maintaining the reverse cold chain to EICRC.

The blood samples (5 ml from adult and 3 ml from children) were collected from each suspected cases in sterile, clean, dry and labeled test tube. The collected blood in test tube was allowed to clot at room temperature. Then the blood in test tube was centrifuged at 3000 rpm for 5 minutes and the serum was separated. After then, the serum samples were transported to EICRC maintaining reverse cold chain. Aliquots for RDT and ELISA were made and stored at 2-8°C until tested.

## **Clinical profile**

A standardized form was used, on the day of admission, to collect information from suspected patients with dengue fever about demographic details, knowledge of dengue, risk factors, breeding places, use of preventive measures and following symptoms: fever, headache, lethargy, muscular pain, rash, retro-orbital pain, joint pain, , nausea, vomiting, abdominal pain and mucosal bleeding.

## **Laboratory Test**

### **Detection of Anti-Dengue IgM by RDT**

#### **Panbio Dengue Duo Cassette (Panbio, Australia)**

##### **Procedure**

All kit components and specimen were equilibrated to room temperature (20-25°C) before commencing the assay. The test device was removed from foil pouch and placed on a flat, dry surface. Using the 10 micro litre (µl) capillary pipette provided, 10 µl serum specimen was drawn and added into the circular sample well. The sample was allowed to absorb entirely into the specimen pad within the circular well. Two drops of buffer was added to the square well holding the buffer bottle vertically and 1 cm above the square well. Test results were interpreted exactly 15 minutes after adding the buffer to the cassette.

### **Detection of Anti-Dengue IgM by IgM-Capture ELISA**

The IgM-capture ELISA was performed according to standard protocol of manufacturer. During the testing procedure, the protocol provided by the Standard diagnostics was strictly followed to achieve high level of accuracy (Appendix-VI for detail procedure).

#### **SD Dengue IgM Capture ELISA Test (Standard diagnostic inc, Korea)**

##### **Procedure**

All reagents were equilibrated to room temperature (20-25°C) before commencing the assay.

##### **Serum Pre-dilution**

Positive control, negative control and patient serum samples were diluted. For this, 10 µl of serum sample/Positive /negative control was diluted to 990 µl of serum diluents (1:100).

##### **Preparation of Antigen**

A bottle of Dengue antigen was diluted using 1.5 ml of the conjugate diluents. The anti-Dengue HRP conjugate was diluted with diluted Dengue antigen in 1:1 ratio. The

mixture solution was gently mixed and left at room temperature (20-25°C) for 60 minutes.

### **Preparation of Tetra Methyl Benzidine (TMB) Substrate**

In a tube, 5 ml of TMB substrate A and 5 ml of TMB substrate B was mixed.

##### **Assay Plate**

The required numbers of micro wells were removed from the foil sachet and were inserted into the strip holder. Five micro wells were required for controls: positive control (P) in duplicate and negative control (N) in triplicate. Within 10 minutes after mixing the monoclonal antibody (MAb) tracer and diluted antigen, 100 µl diluted patient sample and controls were pipetted into their respective microwells of the assay plate. The plate was covered and incubated for 1 hour at 37°C. After incubation, wells were washed five times with diluted wash buffer. The diluted anti-dengue HRP conjugate solution was mixed before transfer. Hundred microlitre of diluted anti-dengue HRP conjugate solution was pipetted into the wells. The plate was covered and incubated for 1 hour at 37°C. The wells were washed five times with diluted wash buffer and 100 µl of mixed TMB solution was pipetted into each well. Timing from the first addition, the plate was incubated at room temperature (15-30°C) for 10 minutes. A blue colour was developed. Then 100 µl of stop solution was pipetted into all wells in the same sequence and timing as the TMB addition. It was mixed well. The blue colour was changed to yellow. The absorbance of each well was read within 30 minutes at a wave length of 450 nm with a reference filter of 620 nm by using Multi ELISA Reader Model 2010 (Anthos, Austria).

### **Interpretation of the Result**

#### **Rapid Test Results Interpretation**

One pink line “C” in the result window suggests no dengue infection, hence the negative test. Two pink lines “C” and “M” in the result window suggests the sample is positive for IgM antibodies to dengue virus. This is indicative of a primary dengue infection. The result is positive even if the “M” line is weak. The results of line “G” indicating IgG antibodies were not interpreted and not included in this study.

## **ELISA Result Interpretation**

A negative result means that DV specific IgM cannot be detected. If a sample is assessed to be positive this means that virus specific IgM has been detected. The test is interpreted either positive or negative on the basis of absorbance with respect to Cut-off value. If absorbance of the sample is greater than cut-off value, the sample is considered positive and if the absorbance of sample is less than cut-off value, the sample is negative.

Cut-off value = mean absorbance of negative controls + 0.300

## **Statistical Analysis**

The collected data was analyzed to find out the relation of dengue with age, sex, occupation, knowledge of dengue, risk factors, breeding place and use of preventive measures. Chi square test and Odds Ratio were determined to find out whether the findings were statistically significant or not. The collected data were analyzed using Statistical package for social science (SPSS) software (version 16.0).

## **Results and Discussion**

During the study period, a total of 261 serum samples were collected, transported and tested by IgM Capture ELISA and Rapid Immunochromatographic Strip test kits (later referred to as RDT).

### **Socio-Demographic Study of Suspected Dengue Cases**

#### **Sex wise Distribution of Suspected Dengue Cases**

Out of 261 suspected dengue cases investigated during the study, 134(51.4%) were males and 127(48.6%) were females. Male to female ratio was 1.05:1.

#### **Age wise Distribution of Suspected Dengue Cases**

The cases under investigation were of the age 1 year to 80 years. The highest numbers of suspected cases 186(71.3%) were from the age group 15-50 years and least numbers of cases 28(10.7%) from the age group of 50 above. 47(18 %) cases belonged to the age group of below 15 years.

## **Profession wise distribution of suspected cases**

Out of 261 serum samples of dengue suspected cases, the highest 90(34.5%) were students and the lowest 21(8%) were farmers.

## **Diagnostic Tests**

Out of 261 serum samples of dengue suspected cases, 49(18.8%) were found to be positive for IgM antibody by IgM Capture ELISA and 40(15.3%) were found to be positive by RDT. (Table 1)

## **Comparison between RDT and IgM-capture ELISA Assay**

Forty-nine (18.8%) of 261 samples were IgM positive by IgM Capture ELISA and 36(73.5%) of the 49 IgM positives by IgM Capture ELISA were also positive by RDT. Four samples positive for IgM by RDT were negative by IgM Capture ELISA and 13 samples positive for IgM by IgM Capture ELISA were negative by RDT.

Two hundred eight samples were Dengue IgM Negative by both IgM Capture ELISA and RDT (Table 2). Compared to IgM Capture ELISA, sensitivity and specificity of RDT is 73.46% and 98.1% respectively with positive predictive value of 90% and negative predictive value of 94.1% (Calculations in Appendix-IV). The Kappa value of the test is 0.77. (Table 2)

## **Age wise Distribution of IgM Positive Case**

In 49 anti-dengue IgM positive cases, the highest number 40(81.6%) of patients were 15-50 years of age and the least; 4(8.2%) cases each were less than 15 and 5(10.2%) were from age group above 50.

The mean age was 28.39 with standard deviation of 17.20. There is no significant association of the anti-dengue IgM positivity with age ( $p=0.124$ ). (Table 3)

## **Sex wise Distribution of IgM Positive Cases**

In 49 anti-dengue IgM positive cases, 33(67.4%) were males and 16(32.6%) were females (male to female ratio=2.06:1). There is significant association of the anti-dengue IgM positivity with sex group ( $p=0.013$ ). (Table 4)

### **Profession wise distribution of positive cases**

Profession wise distribution of positive cases showed the highest number of anti-dengue IgM positive cases in students (6.2%), followed by others (3%) and officers(2.7%) out of total positive cases (Table 5)

### **Clinical Features of Anti-dengue IgM Positive Patients**

Fever 49(100%), headache 32(65.3%), Joint pain 19(33.9), Retro-orbital pain 22(44.9%) and, Muscular pain 21(42.8%) were the major clinical manifestations in the anti-dengue IgM positive cases while Nausea 16(32.6%), vomiting 12(24.5%), Skin rash 11(22.4%), abdominal pain 11(22.4%), Lethargy 15(30.6), and mucosal bleeding 6(12.2) were relatively low in the anti-dengue IgM positive cases. The highest odds ratio 14.651 was found in mucosal bleeding and lowest odds ratio 1.118 in lethargy (Table 6).

### **Relation between Duration febrile illness and IgM Detection**

In 261 suspected cases of DF, the duration of fever less than 5 days was found in 62(23.75%) cases while 199(76.25%) cases was found in 5 days and more. Anti-dengue IgM was detected in only 8(12.9%) cases out of 62 suspected cases with duration of fever less than 5 days and 41(20.6%) cases out of 199 suspected cases with duration of fever of 5 days and more. Out of 49, 16.3% of IgM positive cases were detected in duration of fever less than 5 days and 83.7% of IgM positive cases were detected in duration of fever more than 5 days. Detection rate of anti-dengue IgM positivity and duration of fever was not significantly associated ( $p=0.175$ ). (Table 7)

### **Relation between knowledge of dengue and IgM detection**

In 261 suspected cases, knowledge of dengue was found among 172 (65.9%) and IgM positive in 20 (11.6%) which is 7.7% of total positive cases and knowledge of dengue was not found in 89(34.1%) and IgM positive in 29(32.6 %) which is 11.1% of total positive cases. Detection of anti dengue antibody and knowledge of dengue was found statically significant with p-value of 0.00 (Table 8).

### **Relation between Risk factor and IgM Detection**

In 261 suspected cases, the highest anti dengue positive cases was found in water logging 15(10.9%) followed by travel to endemic area 10(10.3%) and family history of febrile illness 3(3.83%). The highest odds ratio 2.94 was found in travel to endemic area and lowest odds ratio 0.319 in water logging (Table 9).

### **Relation between Breeding place and IgM Detection**

In 261 dengue suspected case, the highest anti dengue positive cases was found in flower pot 19(36.5%), followed by open jar 8(26.6%) and lowest in house drain 10(20%). The highest odds ratio 3.453 was found in flower pot as breeding place and lowest 1.103 in house drain (Table 10).

### **Relation between use of Preventive measure and IgM Detection**

In 261 dengue suspected cases, the highest number of anti dengue positive cases was found in spraying 3(18.75%) followed by cover water container 41(17.6%) and the lowest use of mosquito repellent 3(13.6%) (Table 11).

The present study found that Dengue is one of the most rapidly spreading mosquito-borne viral disease in the world. Some 1.8 billion (more than 70%) of the population at risk for dengue worldwide live in member states of the WHO South-East Asia Region and Western Pacific Region, which bear nearly 75% of the current global disease burden due to dengue (WHO, 2009). Dengue is transmitting in tropical and sub-tropical regions around the world, predominantly in urban and suburban areas. Domestic Dengue Virus Infection (DVI) occurs in more than 100 countries and over 2.5 billion people live in the areas with a risk of dengue virus infection (Gibbons and Vaughn, 2002).

Up to 100 million cases of DF and 500,000 cases of DHF and several thousand deaths are estimated to occur annually worldwide. Dengue is a climate sensitive vector borne disease, which in recent years has become a public health concern.

**Table.1** Calculation of RDT and IgM Capture ELISA Assay

| RDT   | ELISA     |            |               |            |
|-------|-----------|------------|---------------|------------|
|       | Positive  | 36 (a)     | 4 (b)         | 40 (a+ b)  |
|       | Negative  | 13 (c)     | 208 (d)       | 221 (c+ d) |
| Total | 49 (a+ c) | 212 (b+ d) | 261 (a+b c+d) |            |

1. Sensitivity:  $a/(a + c) * 100\% = 73.46\%$
2. Specificity:  $d/(b + d) * 100\% = 98.1\%$
3. Predictive value of positive test =  $a/(a + b) * 100\% = 90\%$
4. Predictive value of negative test =  $d/(c + d) * 100\% = 94.1\%$
5. Percentage of false negative =  $c/(a + c) * 100\% = 26.5\%$
6. Percentage of false positive =  $b/(b + d) * 100\% = 1.9\%$

**Table.2** Diagnostic Tests

| Diagnostic Test   | Total No. of Sample tested | No. of positive cases (%) |
|-------------------|----------------------------|---------------------------|
| IgM Capture ELISA | 261                        | 49(18.8)                  |
| RDT               | 261                        | 40(15.3)                  |

**Table.3** Comparison between RDT and IgM Capture ELISA

| IgM capture ELISA |          |          |          |       |
|-------------------|----------|----------|----------|-------|
| RDT               |          | Positive | Negative | Total |
|                   | Positive | 36       | 4        | 40    |
|                   | Negative | 13       | 208      | 221   |
|                   | Total    | 49       | 212      | 261   |

**Table.4** Age wise Distribution of IgM Positive Case

| Age     | Total no. of suspected case | No. of IgM positive case in suspected and % | % of IgM positive Case n(49) | p-value |
|---------|-----------------------------|---|------------------------------|---------|
| < 15    | 47                          | 4(8.2)                                      | 1.6                          | 0.124   |
| 15 – 50 | 186                         | 40(81.6)                                    | 15.3                         |         |
| >50     | 28                          | 5(10.2)                                     | 1.9                          |         |
| Total   | 261                         | 49(100)                                     | 18.8                         |         |

**Table.5** Sex Wise Distribution of DV Cases

| Sex    | Total no. of suspected case | No. of IgM positive case in suspected and % | % of IgM positive case n(49) | p-value |
|--------|-----------------------------|---|------------------------------|---------|
| Male   | 134                         | 33(67.4)                                    | 12.7                         | 0.013*  |
| Female | 127                         | 16(32.6)                                    | 6.1                          |         |
| Total  | 261                         | 49(100)                                     | 18.8                         |         |

\* Statistically significant

**Table.6** Profession wise distribution of positive case

| Profession   | Total no. of suspected case | No. of Anti dengue IgM positive cases | % of Positive cases out of total population | p-value |
|--------------|-----------------------------|---------------------------------------|---|---------|
| Farmer       | 21                          | 4                                     | 1.6   | 0.899   |
| Business     | 45                          | 6                                     | 2.3   |         |
| Student      | 90                          | 16                                    | 6.2   |         |
| Housewife    | 37                          | 8                                     | 3   |         |
| Officer      | 31                          | 7                                     | 2.7   |         |
| Others       | 37                          | 8                                     | 3   |         |
| <b>Total</b> | 261                         | 49                                    | 18.8  |         |

**Table.7** Clinical Manifestation in Anti-Dengue IgM Positive Cases (n=49)

| Clinical features  | No. of cases | % of IgM positive case | p-value | Odds ratio |
|--------------------|--------------|------------------------|---------|------------|
| Fever              | 49           | 100                    | —       | —          |
| Headache           | 32           | 65.3                   | 0.079   | 1.779      |
| Nausea             | 16           | 32.6                   | 0.104   | 1.750      |
| Vomiting           | 12           | 24.5                   | 0.00*   | 22.59      |
| Retro orbital pain | 22           | 44.9                   | 0.00*   | 7.037      |
| Skin rash          | 11           | 22.4                   | 0.02*   | 3.546      |
| Joint pain         | 19           | 38.8                   | 0.03*   | 2.723      |
| Abdominal pain     | 11           | 22.4                   | 0.284   | 1.515      |
| Lethargy           | 15           | 30.6                   | 0.747   | 1.118      |
| Muscular pain      | 21           | 42.8                   | 0.065   | 1.815      |
| Mucosal bleeding   | 6            | 12.2                   | 0.00*   | 14.651     |

\* \*Statistically significant

**Table.8** Duration of Fever in Relation to IgM Detection

| No. days of onset of fever | Total no. of Suspected case and % | No. of IgM positive cases in suspected and% | % of IgM positive case n(49) | p-value |
|----------------------------|-----------------------------------|---|------------------------------|---------|
| < 5                        | 62(23.75)                         | 8(12.9)                                     | 16.3                         | 0.175   |
| ≥ 5                        | 199(76.25)                        | 41(20.6)                                    | 83.7                         |         |
| <b>Total</b>               | 261(100)                          | 49  | 100                          |         |

**Table.9** Relation between knowledge of dengue and IgM detection

| Knowledge of dengue | Total no. of suspected Case and % | No. of IgM positive in suspected case and % | % of IgM Positive case n(49) | p-value |
|---------------------|-----------------------------------|---|------------------------------|---------|
| Yes                 | 172(65.9)                         | 20(11.6)                                    | 7.7                          | 0.00*   |
| No                  | 89(34.1)                          | 29(32.6)                                    | 11.1                         |         |
| <b>Total</b>        | 261(100)                          | 49  | 18.8                         |         |

\* People with knowledge of dengue have mentioned mode of transmission and at least one symptom of the disease dengue.

\* Statistically significant



**Table.10** Relation between Risk factor and IgM Detection

| Risk factor                       | Total no. of suspected cases at risk and % | No.of IgM positive in suspected case and % | p-value | Odds ratio |
|-----------------------------------|--|--|---------|------------|
| Water logging                     | 138(52.9)                                  | 15(10.9)                                   | 0.001*  | 0.319      |
| Blood transfusion                 | 2(0.7)                                     | 0(0.00)                                    | 0.805   | —          |
| Travel to endemic area            | 27(10.3)                                   | 10(10.3)                                   | 0.01*   | 2.94       |
| Family history of febrile illness | 10(30)                                     | 3(3.83)                                    | 0.354   | 1.91       |

\* Statistically significant

**Table.11** Relation between Breeding place and IgM Detection

| Breeding places | Total no. of suspected cases having breeding place and % | No. of IgM positive in Suspected cases and % | P-value | Odds ratio |
|-----------------|--|--|---------|------------|
| Open jar        | 30(11.5)   | 8(26.6)                                      | 0.239   | 1.685      |
| House drain     | 50(19.1)   | 10(20)                                       | 0.805   | 1.103      |
| Flower pot      | 52(20)   | 19(36.5)                                     | 0.00*   | 3.453      |
| Domestic waste  | 15(5.75)   | 4(26.6)                                      | 0.425   | 1.624      |
| Kitchen garden  | 39(15)   | 11(28.2)                                     | 0.102   | 1.902      |

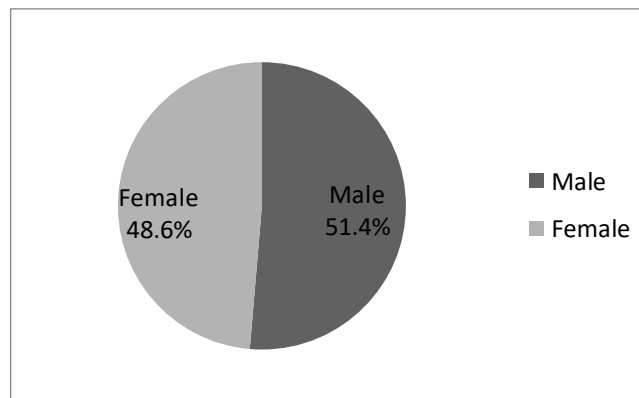
\* Statistically significant

**Table.12** Relation between use of Preventive measure and IgM Detection

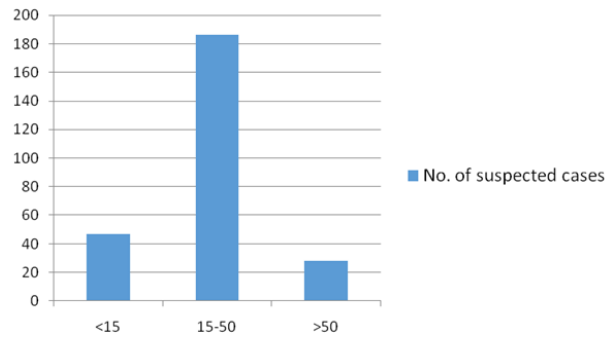
| Preventive measures   | Total no. of suspected cases using preventive measures and % | Total no. of IgM positive in suspected case and % | p-value |
|-----------------------|--|---|---------|
| Cover water container | 233(89.2)  | 41(17.6)  | 0.160   |
| Garbage disposal      | 218(83.5)  | 36(16.5)  | 0.035*  |
| Use of nets           | 228(87.3)  | 38(16.6)  | 0.022 * |
| Spraying              | 16(6.1)  | 3(18.75)  | 0.998   |
| Change stored water   | 224(85.8)  | 37(16.5)  | 0.022*  |
| Mosquito repellent    | 22(8.4)  | 3(13.6)   | 0.519   |

\* Statistically significant

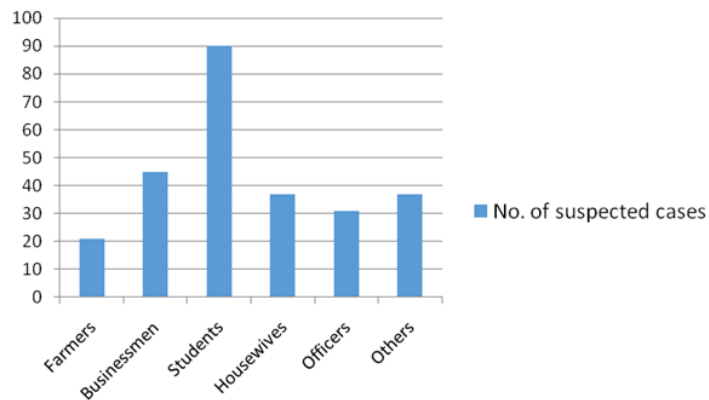
**Figure.1** Sex wise distribution of suspected dengue cases



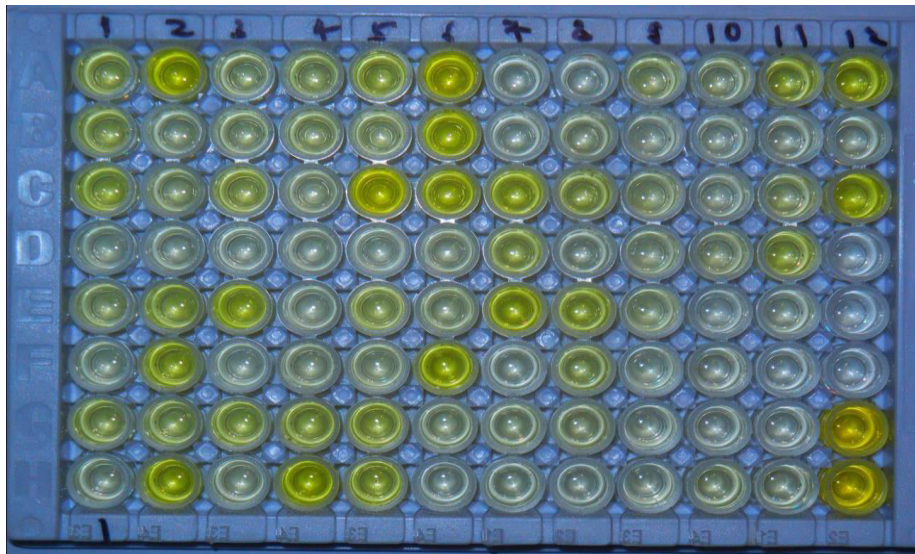
**Figure.2** Age wise distribution of suspected dengue cases



**Figure.3** Profession wise distribution of suspected cases



**Photograph.1** Microtiter wells after addition of stop solution



(A1-A12, B1-B12, C1-C12, D1-D11, E1-E11, F1-F11, G1-G11 and H1-H11 = Serum samples) (D12, E12 and F12 = Negative control; G12 and H12 = Positive control)

**Photograph.2** RDT kit 15 minutes after addition of buffer (Circular well: sample application area, Square well: buffer application area) (C: Control line, M: IgM line, G: IgG line)



The study was a cross-sectional seroepidemiological study done in Narayani Sub Regional Hospital and Bhawani Hospital and Research Centre, Parsha, Birgunj. The present study was carried out during post monsoon period from August to November 2023. Serum samples of 261 clinically suspected patients were analyzed. Out of 261 samples, 49(18.8%) of the tested serum samples were found to be positive for anti-dengue IgM antibody. The sero-positivity of the study was not in accordance with some of the previous findings from Nepal studies carried out by Sah (27.3%) in 2009, Pun (19.3%) in 2009, Poudel (7.2%) in 2009, and Khadka (8.9%) in 2011; Neupane (11.8) in 2012; Subedi (13.73%) in 2012 and Gupta *et al.*, (2013) (29.09%) in 2013 .The change in the positivity rate could be due to variation in geographical distribution. The growth of population and urbanization, increased rate of deforestation, change in environmental conditions may contribute to the increase in prevalence of the disease. The increased rate of migration due to open border might also be the predisposing factor as Terai belt of Nepal is bordered with India. The comparatively higher positive cases might also be due to abundance of Aedes species mosquitoes and the circulation of dengue viral strains.

In conclusion, out of 261 samples collected and tested from two hospitals of parsha district in 2013, 49 were found to be positive for DVI. The samples were tested for anti-dengue IgM antibody by ELISA and RDT and the IgM positivity was 18.8% and 15.38% respectively. The sero-prevalence of dengue has significantly increased so the concerned authority should initiate extensive surveillance of dengue virus infection and

commence an integrated vector control programme in order to abate from a panic viral disease. The diagnosis of DF by molecular and virological tests is a complex process in terms of time and technique in developing countries.

Considering the fact, the present study has attempted to search the, effective serological methods, clinical features, and assessment of risk factor, breeding places and use of preventive measures that would be useful for controlling as well as diagnosis of DF in its early stage. Severity of infection varies according to the serotypes of dengue virus. However, clinical features and serological test can be useful for the diagnosis and assessment of risk factor, breeding places and use of preventive measures in controlling dengue.

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### **Abbreviation**

DENV: Dengue Virus  
RDT: Rapid Diagnostic Test  
ELISA : Enzyme linked Immuno Sorbent Assay  
PCR: Polymerase Chain Reaction

### **Author Contributions**

Shrawan kr. Singh: Investigation, formal analysis,

writing—original draft. Kamlesh Dutta: Validation, methodology, writing—reviewing.

## Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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