Original Research Article

Seroprevalence of Dengue Fever Virus among Suspected Patients in Taiz Governorate-Yemen

Abstract

Background: Dengue Fever virus (DENV) considers one of the most important mosquito-borne viral disease in the world and it is endemic in more than 100 countries.

Objective: This study aimedto determine the seroprevalence of DENV infection among suspectedpatients and to investigate some associated risk factors with dengue fever infection in Taiz governorate, Yemen.

Methods: This study was cross-sectional, descriptive, and experimental, combining the use of a structured questionnaire and analysis of serum samples obtained from 300suspected patients attending at many hospital and clinic centers in Taizduring the period fromJulyto November 2016. The serum samples were tested for anti-dengue immunoglobulin (IgM) and (IgG) by Enzyme-linked Immunosorbent Assay (ELIZA).

Results: Out of 300 suspected febrile cases, it was found that 49(16.3%),68(22.7%), and 17(5.7%) cases were showed positive for the IgM, IgG, and both IgM and IgG antibodies, respectively, while 166 (55.3%) cases were negative.The incidence rate was more in males than in females. The most affected age group with dengue fever infection were (21–30) years. Dengue Fever was more frequent among patients coming from the urban area, having secondary school, and low-income status people. Also, there was statistical significant between DENV infections with a place of residencyand gender (P<0.05) and not-statistical significance between DENV infections and other factors (P>0.05).**Inconclusion**,Taiz governorate become one of the endemic governorates in Yemen particularly the Taiz city which should be brought to the attention of public health authorities

Keywords: Dengue fever virus,IgG,IgM, Seroprevalence,Taiz governorate, Yemen.

INTRODUCTION

DengueFever virus(DENV) is one of the most important mosquito-borne viral disease in the world and it is endemic in more than 100countries, with an estimated 100 million infected cases and about 25,000 deaths per year worldwide**1.**DENV is an enveloped, positive-sense, single-stranded RNAgenome that belongs to the family Flaviviridae, genus Flavivirus[2]**.**

Dengue fever is caused by one of the four serotypes of dengue viruses (DENV1-4). These types of viruses are transmitted via infective female mosquitoes, namely *Aedesegypti* (principal vector) and *Aedesalbopictus*, through the bites or blood meals on human hosts [3]. Direct person-to-person transmission has not been documented. Although a few case reports have been published on the transmission of DENV through exposure to DENV-infected blood, organs, or other tissues from blood transfusions, solid organ or bone marrow transplants[4,5].

Dengue virus causes primary and secondary infections. Primary infection is an acute feverish illness known as Dengue Fever (DF) which mostly eliminate around seven days by a composite immune response, while the secondary infection is additional rigorous and causes Dengue Hemorrhagic Fever(DHF) or Dengue Shock Syndrome (DSS)[6,7].The majority of deaths that result from dengue infectionresult from Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) [8].

In Yemen, the early first recorded of dengue-like epidemics inAden was reported by Hirsch between 1870 and 1873 years[9]. Also,the prevalence of dengue fever was recorded in Al-Hudidah governorate in 1954 which affected 98% of the studied population [10].After that dengue fever became an epidemic in the coastal planes of the Tehama (Hudidah), then outbreaks increased since 2005 and the disease has spread to new governorates [11].

All three dengue viral serortyp1-3 were confirmed to be in Yemen by using PCR, but Dengue virus type 2 was the most predominant serotype in Yemen [12].Interestingly DENV serotype4 wasidentified and confirmedthe first time in Al-Hudidah-Yemen byAlahdal*et al.*[13].

In the Taiz governorate, the infection rate varied from year to year.Recently,WHO[14]documented an extreme prevalence in cases of dengue fever in Taiz governorate were reached to1328suspected cases compared with last years. But seroprevelance of DENV is not well documented so far. Therefore, this study designed to determine the seroprevalence of dengue fever and to reveal some possible risk factors associated with DENV infection in Taiz governorate, Yemen.

MATERIALS AND METHODS

Study area

Taiz governorate is situated in the southwestern part of the Republic of Yemen between latitudes (12-14o) North of the equator and between longitudes (43-45o) East of GMT. Taiz is the third largest governorate in Yemen, itholds the first rank in terms of the population which rate 2,393,425 according to the census results for the year (2004) [15]. Administratively, Taiz is among the largest governorates as it includes 23 districts in its frame. The climate of Taiz is characterized by diversity, where their highland parts are cold in winter and mild in summer, while low parts are mild in winter and warm to hot relatively in summer. The climate in the western parts of the governorate is the coastal desert climate that is warm in winter and hot in summer, and the average temperature in the governorate is up (21°C). The rain falls in the summer on all parts of the governorate and in some districts in the winter[16].

Study design

This is a cross-sectional, descriptive was conducted fromJulyto November 2016, performed on suspected patients suffering undifferentiated fever (age range, 1 to 65 years) attending many hospitals and health centers at nine main locations in the Taiz governorate, which most patients coming seeking medical care.

Ethical approval

Approval for this study was obtained from the Ethical Review Committee in the Biology Department, Faculty of Science, Sana'a University.

Data collection

A structured questionnaire was designed to collect required data from suspected patients regarding socio-demographics and risk-related data. Thequestionnaire was filled for each participant via face-to-face interview by a researcher to avoid any misunderstanding and confirm the accurate collection of data properly.

Sample collection

Five-mL blood sample was collected from 300suspected patients suffering undifferentiated feverby venipuncture, transferred into a sterile anticoagulant-free sterile bottle, and allowed to clot. The clotted blood sample was centrifuged (3000 rpm, 5 min), andthe serum (the supernatant) was put in separate Eppendrof tubes with a specific study number (SNO) transferred inside a cooling box and stored at -20°C until required for use.

Figure 1: Map of Yemen and Taiz governorate

SerologicalAssay

Samples of serum were tested for DENV-specific IgG and IgM antibodies by using IgG and IgM DRG Immunodiagnostic Kits(GmbH Germany)that performed by Enzyme-Linked Immunosorbent Assay (ELISA)(Absorbance Microplate Reader/ELIZA-IRE96,SFRI, French)[17].

Statistical analysis

The obtained data were analyzed using version 18.0 SPSS (Statistical Package for Social Science). A significant difference between the proportions and the groups or variables was determined byChi-square test (≥3.9 considered significant) and*P*-value (< 0.05 considered significant).

RESULTS

In a total of 300 participants enrolled in this study, 202 (67.3%) were malesand 98 (32.7%) were females. The highest participant groups in the present study were patients aged between (21-30) years with an average of 105 (35%). Most suspected patients 195 (65%) were living in urban areas. The suspected patients with secondary education levels were more than the third 113 (37.7%) of the total population in this study.On the other hand, more than half of the respondents 160 (53.3%) were of low-income status (Table 1).

Table 1: Characteristics of study cases

Variable		**Number (%)**	**Variable**		**Number (%)**
Gander	**Male**	202 (67.3)	**Education level**	**Illiterate**	46 (15.3)
	Female	98 (32.7)		**Primary**	56 (18.7)
Age	**1 – 10**	24 (8)		**Secondary**	113 (37.7)
	11 – 20	58 (19.3)		**University**	85 (28.3)
	21 – 30	105 (35)	**Income Status**	**Low Status**	160 (53.3)
	31 – 40	64 (21.3)		**Med Status**	118 (39.3)
	41 – 50	29 (9.7)		**High Status**	22 (7.3)
	> **50**	20 (6.7)			
Residence	**Rural**	105 (35)			
	Urban	195 (65)			

Out of 300 suspected febrile cases, 49 (16.3%) showed positive results for the IgM antibodies (acute infection) and 68 cases (22.7%) were positive for the IgG antibodies (chronic infection), and 17 cases (5.7%) were positive for both IgMandIgG (acute and chronic infection), while 166 (55.3%) cases were negative for anti DENV antibodies (Figure 2).

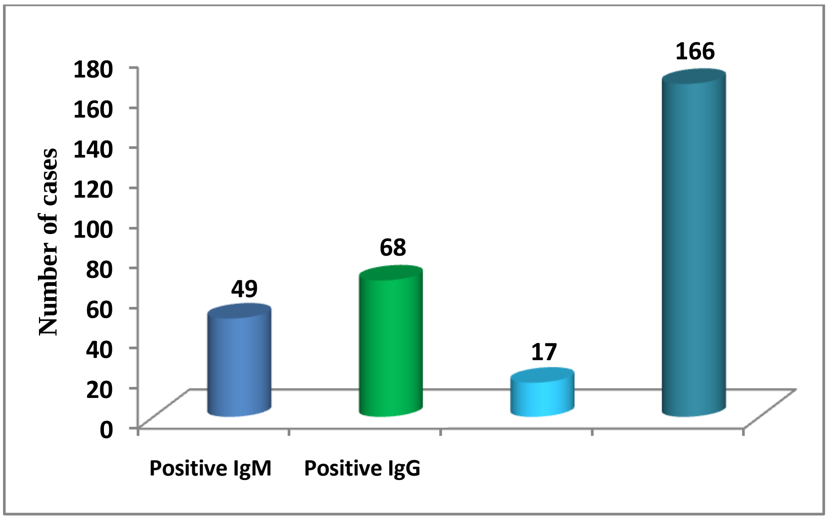

Figure 1: The positive and negative of anti DENV antibodies

In the present results, the DENV IgM, IgG, and both IgM and IgGseropositivitywere mostly observed in male patients, 39 (19.3%), 53 (26.2%), and 14 (6.9%), respectively, while the female patients were lower from that 10 (10.2%), 15 (15.3%), and 3 (3.1%), respectively. Also, thestatistical analysis showed a significant association between the gender and seroprevalence of DENV IgM and DENV IgG (*P*< 0.05) (Table 2).

In the current work, the higher prevalence rate of DENV IgM, IgG, and both (IgM and IgG) seropositivitywere observed among patients aged from 21-30 years with 22 (21%), 29 (27.6%), and 8 (7.6%), respectively. While the lowest from those was among (> 50) years, 2 (10%), 3 (15%) and 0 (0 %), respectively. There was an association between age groups and seroprevalence of DENV was not significant (*P*>0.05) (Table 2).

The result regarding the resident area, it was found that 19.5% of positive anti-IgM, 26.2% of positive anti-IgG, and 8.2% of positive anti-IgM and anti-IgG antibodies were reported among patients coming from the urban area. On other hand, the seropositivity of DENV antibodies among cases living in the rural area was 10.5% for IgM,16.2% forIgG, and1% for both IgM and IgG. Also, there was a statistically significant association between the place of residence andseroprevalence of DENV antibodies(*P*< 0.05) (Table 2).

DENV IgM, IgG, and both (IgM and IgG) seropositive were the highest ratesamong participants who have secondary education level with rate 22 (19.5%), 31 (27.4%), and 7 (6.2%), respectively, whereas the lowest rate was found among illiterate participants with 5 (10.9%), 7 (15.2%) and 2 (4.3%), respectively. The result showed no significant association between any education levels and seroprevalence of DENV (P > 0.05) (Table 2).

Table 2 shows the highest rate of DENV seropositivity of IgM, IgG, and both IgM and IgG antibodies were recorded among cases with low-income status when compared to the lowest seropositivity of DENV antibodies among cases with high-income status.

Table 2: Distributions of anti-DENV with socio-demographical characteristic of the suspected patients

Variables		**Number of cases**	**IgMpositive**		**IgG positive**		**IgM and IgG positive**	
		No. (%)	**No. (%)**	***P*-value**	**No. (%)**	***P*-value**	**No. (%)**	***P*-value**
Gander	**Male**	202 (67.3)	39 (19.3)	**0.04**	53 (26.2)	**0.03**	14 (6.9)	**0.2**
	Female	98 (32.7)	10 (10.2)		15 (15.3)		3 (3.1)	
Age (years)	**1 – 10**	24 (8)	3 (12.5)	**0.7**	4 (16.7)	**0.7**	1 (4.2)	**0.8**
	11 – 20	58 (19.3)	9 (15.5)		12 (20.7)		3 (5.2)	
	21 – 30	105 (35)	22 (21)		29 (27.6)		8 (7.6)	
	31 – 40	64 (21.3)	9 (14.1)		14 (21.9)		4 (6.3)	
	41 – 50	29 (9.7)	4 (13.8)		6 (20.7)		1 (3.4)	
	>50	20 (6.7)	2 (10)		3 (15)		0 (0)	
Residence	**Rural**	105 (35)	11 (10.5)	**0.04**	17 (16.2)	**0.04**	1 (1)	**0.01**
	Urban	195 (65)	38 (19.5)		51 (26.2)		16 (8.2)	
Education level	**Illiterate**	46 (15.3)	5 (10.9)	**0.6**	7 (15.2)	**0.6**	2(4.3)	**0.9**
	Primary	56 (18.7)	8(14.3)		11		3(4.5)	

					(19.6)			
	Secondary	113 (37.7)	22(19.5)		31(27.4)		7(6.2)	
	University	85(28.3)	14(16.5)		19(22.4)		5(4.8)	
Income Status	**Low**	160 (53.3)	30 (18.8)	**0.4**	41 (25.6)	**0.2**	10 (6.3)	**0.4**
	Med	118 (39.3)	17 (14.4)		25 (21.2)		7 (5.9)	
	High	22 (7.3)	2 (9.1)		2 (9.1)		0 (0)	

χ^2Chi-square ≥3.8; ***P***<0.05 (significant)

DENV IgM, IgG, and both (IgM and IgG) seropositivity case the most effective breeding sites of mosquitoes were the bogs that were 31 (19.6%), 42 (26.6%), and 11 (6.9%) of infected cases. The second source was trashes 36 (18.2%), 46 (23.4%) and 12 (6.1%), then open drums 24 (17.1%), 31 (22.1%) and 10 (7.1%), followed by, open sewage 10 (16.7%), 13 (21.7%) and 2 (3.3%). Next, pools 6 (13.6%), 9 (20.5%) and 4 (9.1%) respectively. The lower abundant site was tires which recorded 7 (12.5%), 10 (17.9%) and 3 (5.4%), respectively of the breeding sites.There was no significant association between the seroprevalence of DENV and all breeding sites of mosquitoes(*P*> 0.05) (Table 3).

Table 3: Seroprevalence of DENV antibodies according to breeding sites of mosquitoes.

Breeding sites	**Examined Cases**	**IgM positive**			**IgG positive**			**IgM and IgG positive**		
	No. (%)	**No. (%)**	X^2	***P*-value**	**No. (%)**	X^2	***P*-value**	**No. (%)**	X^2	***P*-value**
Bogs	159 (53)	31 (19.6)	2.4	0.1	42 (26.6)	2.7	0.1	11 (6.9)	0.9	0.3
Open Sewage	60 (20)	10 (16.7)	0.01	0.9	13 (21.7)	0.04	0.8	2 (3.3)	0.7	0.3
Pools	44 (14.7)	6 (13.6)	0.3	0.6	9 (20.5)	0.1	0.7	4 (9.1)	1.2	0.3
Open drums	140 (46.7)	24 (17.1)	0.1	0.7	31 (22.1)	0.04	0.8	10 (7.1)	1.1	0.3
Trash	197 (65.7)	36 (18.2)	1.5	0.2	46 (23.4)	0.1	0.7	12 (6.1)	0.2	0.7
Tires	56 (18.7)	7 (12.5)	0.7	0.4	10 (17.9)	0.9	0.3	3 (5.4)	0.01	0.9

χ^2Chi-square ≥3.8; ***P***<0.05 (significant)

In the present finding,the most of infected cases that observed positive forIgM, IgG, and both (IgM and IgG) antibodies were recorded inTaiz city with 31 (19.4%), 43 (26.9%) and 10 (6.9%), followed by Al-Barh 8 (17.7%), 11 (24.4%) and 3 (6.7%), then Al-Rahedah 4 (16.7%), 5 (20.8%) and 2 (8.3%), after that, Al-Makha 2 (12.5%), 3 (18.8%) and 1 (6.2%). Next, Al-Demna 2 (10%), 4 (20%) and 1 (5%), respectively, the lowest of that wereHajdah 1 (10%) for IgM, 2 (20%) for IgG and Mawyah 1 (10%) for IgM. While two locations were free of infection namely Al-Turbah and Al-Nashamah which no recorded any cases for IgM orIgGseropositivity (Table 4).

Table 4: The distribution of DENV antibodies according to sampling location

Area	Participants No. (%)	IgM positive No. (%)	IgG positive No. (%)	IgGandIgM positive No. (%)
Taiz city	160 (53.4)	31 (19.4)	43 (26.9)	10 (6.9)
Al-Barh	45 (15)	8 (17.7)	11 (24.4)	3 (6.7)
Al-Rahedah	24 (8)	4 (16.7)	5 (20.8)	2 (8.3)
Al-Makha	16 (5.3)	2 (12.5)	3 (18.8)	1 (6.2)
Al-Demna	20 (6.7)	2 (10)	4 (20)	1 (5)
Hajdah	10 (3.3)	1 (10)	2 (20)	0 (0)
Mawyah	10 (3.3)	1 (10)	0 (0)	0 (0)
Al-Turbah	10 (3.3)	0 (0)	0 (0)	0 (0)
Al-Nashamah	5 (1.7)	0 (0)	0 (0)	0 (0)
Total	**300 (100)**	**49 (16.3)**	**68 (22.7)**	**17 (5.7)**

DISCUSSION

The findings of this study revealed that the overall seroprevalence of DENV antibodies among suspected patients was 134 cases (44.7%). 49 cases (16.3%) were positive for acute DENV infection (IgM positive) and 68 (22.7%) of suspected cases were found positive for chronic DENV infection (IgG positive), The cases that were positive for both acute and chronic infection (IgM and IgG positive) were 17 (5.7%), while 166 (55.3%) of suspected cases were negative for dengue fever virus.

Interestingly, in this study, the level of DENV chronic infection (IgGseropositivity) was found (22.7%) more than acute infection (16.3%) and both (5.7%). These results were similar to a study inthe Shabwah governorate by Al-Moyed*et al.*[18]who showed that 438 (53.5%) of cases were positive for DENV antibodies. Similarly,Abdullah *et al.* [19] found that 179 cases were positive for IgM antibody (42% of all suspected specimens) and 262 cases were positive for IgG antibody (61.6%). Also, 96 specimens were positive for both (22.5%). However, 83(19.5%) of suspected cases were negativefor DENV antibodies.

The high prevalence of dengue IgG among suspected cases in this study may be attributed to previous exposures and endemicity of infection, especially in Taiz city, which may be increasing the risk of complicated dengue infection through a phenomenon known as antibody-dependent enhancement depending on the number of DENV serotypes that circulating in the governorate. This observation is an agreement witha study byMadani*et al.*[20].

However, the war from 2015 until nowin Yemen particularlyin Taiz that lead to limited primary health care services, lack of water supply systems, sanitation services, and insufficient control on mosquitos breeding sites facilitating the spread of endemic diseases such as dengue fever.

Furthermore, the distribution of DENV IgM, IgG, and both (IgM and IgG) seropositivity in Taizmostly observed in male cases, (19.3%), (26.2%) and (6.9%), respectively. While the female cases were lower from that (10.2%), (15.3%) and (3.1%), respectively.This result was supported withsimilar previous reports in different countries that noticed that the males were more affected by fever infection than femalesby Bin Gluth*et al.* [21], Madani*et al.*[20], andAbdullah *et al.*[19] in Yemen; Ayyub*et al.*[22] in Saudi Arabia, Abdelhalim*et al.*[23] in Sudan.

The possible reason for the high number of dengue infected cases among males due to the habit of males in the summer season, they did not cover their body whether at home or outside, spend more time outdoor and they have traveling history to that area where the dengue incidence is high. These habits make them more exposed to the bite of *Aedesaegypti*.

In the present study, it was revealed that the most susceptible age group for DENV infection were (21-30) years which showed a higher prevalence of dengue infection (IgM=21%, IgG=27.6% and both =7.6%). While, the less infected category with dengue infection was the older people aged (> 50) years with an average (10%), (15%) and (0%) for IgM, IgG, and both, respectively. Similar observations were also reported in Yemen byMadani*et al.*[20]and Qassim[24], in India by Akula and Kammili[25], and Pakistan by Muhammad *et al.* [26]. This result suggesting that the individuals in these age groups were more actively outdoor during the day which increased their chances of exposure to the infective DENV vector bite.

According to the present study, the relationship between the dengue fever seropostivity and education levels which found higher in people with secondary education levels compared with lower among illiterates participants. This finding is similar to a study by Abdullah *et al.*[19]conducted in Yemen. This finding may reflecttheir outdoor activity during the day, for playing, schooling, or picnic that increased their chances of exposure to the infective DENV vector bite.

The prevalence of dengue fever infection was noted significantly correlated with place of residence (rural or urban) areas. The prevalence rate of dengue fever infection in the urban area was more than the rural areas. This finding is in agreement with earlier studies in Yemen by Abdullah *et al.*[19] and Bin Gluth*et al.*[21].

In the present study, the income statues were found non- significantly associated with dengue infection. But Dengue IgM, IgG and both seropositivity, were found highest with low-income statues cases (4-7 $ daily), it was rated (18.8%), (25.6%) and (6.3%), respectively. Whereas, the less infected people were with high-income statues (>20 $ daily), which rated (9.1%), (9.1%) and (0%) for IgM, IgG, and both, respectively. Similar results were reported byAl-Hemiree[27] and Abdullah *et al.*[19]in Yemen and byMuhammad*et al.* [26]in Pakistan.

Furthermore, in this workthe dengue mosquitoes breeding sites were found positive factors related to dengue IgM, IgG, and both seropositivity. The bogs were the most breeding that associated with most of the suspected cases comparedto the lowest rate among the other factor such as trash, open drums, pools, open drainage and the tires. This result is an agreement with Abdullah *et al.*[19] who noted that the highest breeding site factor was the bogs which rated (92.2%), while the lowest factor was the tires with an average (26.3%).

Interestingly, the distribution of dengue fever results according to districts revealed that the of the most hotspot of dengue fever virus infection in Taiz governorate was concentrated in the city of Taiz, followed by Al-Barh, Al-Rahedah, Al-Makha, Al-Demna, Hajdah, and Mawyah. Whereas two districts namely Al-Turbah and Al-Nashamahwere found free from DENV infection that may be due to climate change and the lack of suitable conditions for themosquitos breeding cycle in these areas.

The low infected cases were found Al-Makha during this study that the infected case was low maybe contributing to the war most people replacement to another area within the governorate most infected case in Al-Barhwhich were coming from Alhodidah and Al-Makha.

CONCLUSION

In conclusion, the high prevalence of DENV antibodies in Taiz is becoming one of the most endemic governorates in Yemen which should be brought to the attention of public health authorities.Warm climate, rainfall also the war since 2015 until now contributed todestroyingYemen's healthcare, presence of breeding sites, lack ofwater supply systems and mosquito control measure, low-income status, and insufficient sanitation systems are the reasons that attributed to the increase of suspected cases of DENV among study area. Therefore, continuoussurveillance for outbreaks of DENV infection required to identify early andin order to prevent and control the spread of infections among the community.

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