

Risk Factors Associated with a Dengue Fever Outbreak in Mille Woreda, Ethiopia, 2023: Evidence from a Case-Control Study

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ABSTRACT

Background: Dengue fever is a mosquito-borne viral infection caused by the dengue virus and is endemic to most tropical and subtropical parts of the globe. In Ethiopia, dengue fever initially appeared in 2013 and became a major public health problem. The objective of this study was to examine an outbreak of dengue fever in Mille Woreda, Zone One, Afar Region, Ethiopia, by reporting its time, place, and person distribution, and identifying the risk factors involved.

Methods: We carried out a descriptive study followed by a case-control study with a ratio of 1:2. We employed the case definition of the World Health Organization to make cases in Mille Woreda from April 13 to May 7, 2023. Controls were included from the same town who were not showing symptoms of dengue fever. Serum samples were subjected to reverse transcription polymerase chain reaction (RT-PCR) for detection of the dengue virus and determination of serotypes. A standard questionnaire was used to collect socio-demographic, behavioural, and environmental characteristics. Multivariate logistic regression was used for analysis.

Results: We identified 57 cases and 114 controls. The overall attack rate was 367/10,000. Eight out of ten of the serum samples were positive for dengue virus serotype DEN-3. In multivariate analysis, never having heard of dengue fever (Adjusted odds ratio [AOR]: 3.76, 95% CI: 1.46–9.65), not knowing how it's transmitted (AOR: 2.88, 95% CI: 1.14–7.25), and long-lasting insecticidal net use (AOR: 6.83, 95% CI: 2.63–17.7) were statistical risk factors. Wearing long sleeves (AOR: 0.11, 95% CI: 0.04–0.28), on the other hand, was a protective behavior.

Conclusion: It was confirmed that dengue fever outbreak occurred in Mille Woreda. Data from this study offer evidence-based data on modifiable risk factors that have led to the outbreak. Public health education, intensified vector control intervention, and strengthened dengue surveillance systems are advised to avert future outbreaks.

Keywords

Case-control study, Dengue fever, Outbreak, Risk factors, Mille.

Transcription Polymerase Chain Reaction, SPSS: Statistical Package for the Social Sciences, WHO: World Health Organization.

List of Abbreviation

AOR: Adjusted Odds Ratio, CI: Confidence Interval, DF: Dengue Fever, DENV: Dengue Virus, EPHI: Ethiopian Public Health Institute, IgG: Immunoglobulin G, IgM: Immunoglobulin M, K.M: Kilometer, LLINs: Long-Lasting Insecticidal Nets, PCR: Polymerase Chain Reaction, PHEM: Public Health Emergency Management, RNA: Ribonucleic Acid, RT-PCR: Reverse

Background

Dengue fever (DF) is a mosquito-borne viral infection caused by the dengue virus, which belongs to the genus *Flavivirus* and the family *Flaviviridae* [1]. There are four antigenically distinct but closely related serotypes of the virus: DENV-1, DENV-2, DENV-3, and DENV-4 [2]. Infection with one serotype confers lifelong immunity against that specific type; however, it does not provide

cross-protective immunity to the others, making individuals susceptible to reinfection with a different serotype [3]. Transmission occurs primarily through the bite of an infected female *Aedes* mosquito, mainly *Aedes aegypti* [4]. Less commonly, dengue can also be transmitted via blood transfusion, organ transplantation, or vertically from mother to fetus [5]. The mosquito vector becomes infected after biting a viremic person [6]. Clinical symptoms usually manifest between 3 to 14 days following infection and include high fever, severe headache, myalgia, arthralgia, vomiting, and a characteristic rash. While there is no specific antiviral treatment for dengue, most cases resolve within 2 to 7 days with supportive care. The dengue vaccine (Dengvaxia) is licensed for children aged 9–16 years with prior confirmed dengue infection living in endemic areas [7]. Preventive strategies focus on avoiding mosquito bites and vector control [8]. Early detection and appropriate clinical management are critical for minimizing severe outcomes and curbing further transmission [9]. Dengue fever is endemic in many parts of the tropical and subtropical world, putting more than half of the global population at risk [10]. It is estimated that over 3.6 billion people in more than 100 countries—particularly in Asia, the Americas, and Africa—are vulnerable to dengue infection, with approximately 390 million cases occurring annually [3]. **In Africa, the true burden of dengue remains underrecognized, although outbreaks in various countries highlight the growing threat of dengue transmission on the continent.**

In Ethiopia, dengue fever was first reported in Dire Dawa in 2013 [11]. With subsequent outbreaks identified in Afar and Somali Regions from 2014 to 2017 [12]. Dire Dawa experienced recurrent outbreaks between 2013 and 2017 [13]. Sporadic cases and outbreaks have continued to be reported across Ethiopia, signaling a growing public health concern.

On April 14, 2023, a suspected dengue fever outbreak was reported by the Mille Woreda public health surveillance officer to the Afar Regional Public Health Emergency Management (PHEM) Directorate and the Ethiopian Public Health Institute (EPHI). In response, EPHI deployed a multidisciplinary investigation team on April 19, 2023.

Therefore, this study aimed to investigate the outbreak by describing its distribution by time, place, and person, and to identify risk factors associated with dengue transmission in Mille Woreda, Zone One, Afar Region, Ethiopia. The findings aim to inform targeted control strategies and improve preparedness for future outbreaks in resource-limited settings.

Materials and Methods

Study Area

The Investigation was conducted in Afar Region, Administrative Zone one (Awsa Rasu), Millie woreda, Millie kebele 01 which is located 567 K.M from the capital city Addis Ababa. Based on the information we got from the local authority; the district has 116,481 total populations, of which are 64, 065 were males and females account 52, 416. The woreda has a total of 12 kebeles, 5 health centers and 10 health posts which delivery health care

service to the community.

Study Design and Period

Descriptive cross-sectional studies from line listing were conducted to describe the outbreak in terms of place, person and time followed by unmatched case-control study with one to two ratios from April 13, 2023, to May 7, 2023.

Sample Size Determination

We calculated the sample size using Epi-info statistical computing software with a power of 80%, odds ratio of 2.776 for no use of LLINs, exposed control percentage of 23.6% [11], and the case-to-control ratio of 1:2. The total sample size was 171, with 57 cases and 114 controls.

Source population

All residents of Mille woreda who were attending the health facility.

Study Population

Subset of source population who fulfil the inclusion criteria for dengue fever disease

Inclusion and Exclusion criteria

Inclusion criteria

Cases were either confirmed or epidemiologically linked to Dengue Fever cases while controls were all people without Suspected Dengue fever symptoms. All confirmed or epidemiologically linked cases of dengue fever found in Health facilities from April 13-May 7, 2023 were included in the study. For the Controls, a resident of Mille woreda who was a neighbor to a case and who did not develop signs and symptoms of Dengue fever was included in the health facility was enrolled.

Exclusion criteria

Suspected Dengue fever patients who were critically ill and controls who was not a permanent resident of Mille woreda were excluded from the study.

Variables

Dependent variable

Dengue fever status of participants during the study period.

Independent variable

Gender, Age, Occupation, Educational level, Marital Status, Bed net utilization, traveling history to the affected sites, house hold spraying in the last six months, presence of open water container, close contact with ill Person in the Last 2 weeks, type of cloths.

Case Definition and Selection of Cases and Controls

Case Definition: Patient with sudden onset of fever and the presence of one or more signs and symptoms of dengue fever: nausea, vomiting, rash, aches/ headache, retro-orbital pain, joint pain, myalgia, arthralgia, Tourniquet test positive, leukopenia (a total white blood cell counts of two centimeters, increasing hematocrit concurrent with rapid decrease in platelet count.

Suspected: Any patient with fever and one or more of Dengue fever signs and symptoms that having epidemiologic linkage.

Probable: Any patient with fever and one or more of Dengue fever signs and symptoms and detection of anti-DV IgM in serum sample.

Confirmatory: Detection of Dengue virus nucleic acid in serum, plasma, blood, cerebrospinal fluid, other body fluid or tissue by validated laboratory test method such as reverse PCR reaction, immune fluorescence or immunoassay to detect seroconversion of anti-DV IgM or anti-DV IgG.

Epidemiologic Link: Travel to a dengue endemic area or presence at location with ongoing outbreak within the previous two weeks of the onset of an acute febrile illness or dengue or association in time and place (e.g., household member, family member, classmate, or neighbor) with a confirmed or probable dengue case.

Cases- Are individuals, which fulfils the above criteria and all reported cases were included in the study.

Controls- These are individuals, who do not fulfill the above criteria and selected from a similar village where cases were identified.

Laboratory Investigation

Blood samples were collected from 10 patients who met the case definition and centrifuged at Mille primary hospital before being shipped to the National Reference Laboratory of the Ethiopian Public Health Institute (EPHI). The serum specimens were shipped with their accompanying case investigation forms to the National Reference Laboratory of the Ethiopian Public Health Institute (EPHI). Appropriate implementation of triple packaging of the samples to maintain the cold chain was ensured and each completed case reporting form was also included. The serum was then subjected to ribonucleic acid (RNA) extraction using a QIAGEN RNA extraction mini kit (QIAamp Viral RNA Mini Kit). Amplification was conducted using the Invitrogen super script III platinum one-step qualitative RT-PCR Kit. The total reaction volume was 25ul and composed of 10ul of the RNA extract elute and 15ul of the prepared master mix from the Invitrogen kit (Super Script III). Each sample was tested for dengue viruses using the triplex real-time RT-PCR assay [14].

Data Collection

We reviewed the medical records of the cases at the Millie Health Center and the laboratory findings of the cases at the National Reference Laboratory. Epidemiological data were collected through face-to-face interviews using a structured questionnaire prepared in English with the help of local guides and translators. Laboratory specimens were collected by the investigator. The questionnaire was divided into three main areas: demographic information, clinical information, laboratory specimen information, knowledge assessment, and disease exposure information. To verify consistency, the questionnaire was pretested a day before

data collection.

Statistical Analysis

The data from the questionnaires were entered into a Microsoft Excel spreadsheet, edited, and coded. Statistical Package for the Social Sciences (SPSS) version 23.0 was used for analysis. We performed a logistic regression analysis to compare the exposure cases and controls. Each variable with a P-value ≤ 0.05 was kept in the multivariable analysis. A P-value of less than 0.05 at the 95% confidence interval was considered significant.

Result

Descriptive Analysis

As shown in Figure 1, The dengue outbreak started on April 13, 2023. The epidemic curve showed multiple peaks that indicated a propagated outbreak.

The first case involved a 50-year adult male patient from Kebele 01. He presented with headache, high-grade fever, vomiting, and periorbital pain and visited the primary hospital on April 13, 2023. No cases have been reported after May 7, 2023. A total of 498 cases were reported in Mille woreda kebele 01 during the outbreak period from April 13 to May 7, 2023. Of the total registered cases, 324 (65%) were males with a mean age of the cases being 30 years (range, 3–55 years). All patients were treated as outpatients, and there were no deaths during the dengue fever outbreak. Among the 12 kebeles in the district, dengue fever was detected on only one kebele, with a total population of 38, 658 and male accounts for 19, 754 and female for 18,904. The overall crude attack rate is 367 per 10,000 people. The sex-specific attack rate for this outbreak was 16 dengue fever cases per 1000 population for males and 9 dengue fever cases per 1,000 population for females. The majority of case 365 (73%) were age group 15 to 45 years, followed by age greater than 45 years cases 87 (17%) and age group 5 to 14 cases 42 (8%) and the rest only one case were age less than 5 years old (Table 1).

Table 1: Dengue fever case distribution by sex and age group in Mille woreda, Afar region 2023.

Characteristics	Grouping	Number	Percentage
Sex	Male	324	65%
	Female	174	35%
	Total	498	100
Age group	<5 years	4	0.8%
	5-14	42	8%
	15-45	365	73.2%
	> 45	87	17.4%
	Total	498	100

As shown in Figure 2, all 498 cases had a fever (100%). Other signs and symptoms were headache (84.5%), myalgia (81%), joint pain (47%), vomiting (51.4%), retro-orbital pain (35.1%), and nasal bleeding (17%).

Laboratory Investigation

Laboratory tests were not available at the outbreak site, and it was generally not feasible to send samples to the Ethiopian Public

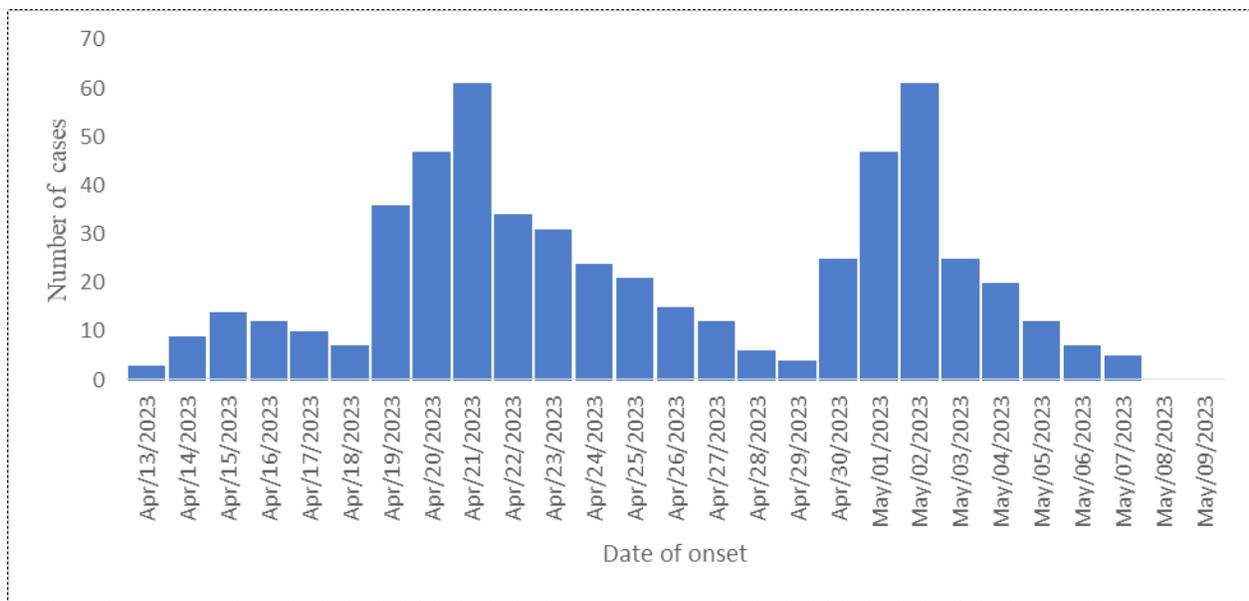


Figure 1: Epidemic curve of Dengue Fever outbreak by date of onset Millie woreda 2023.

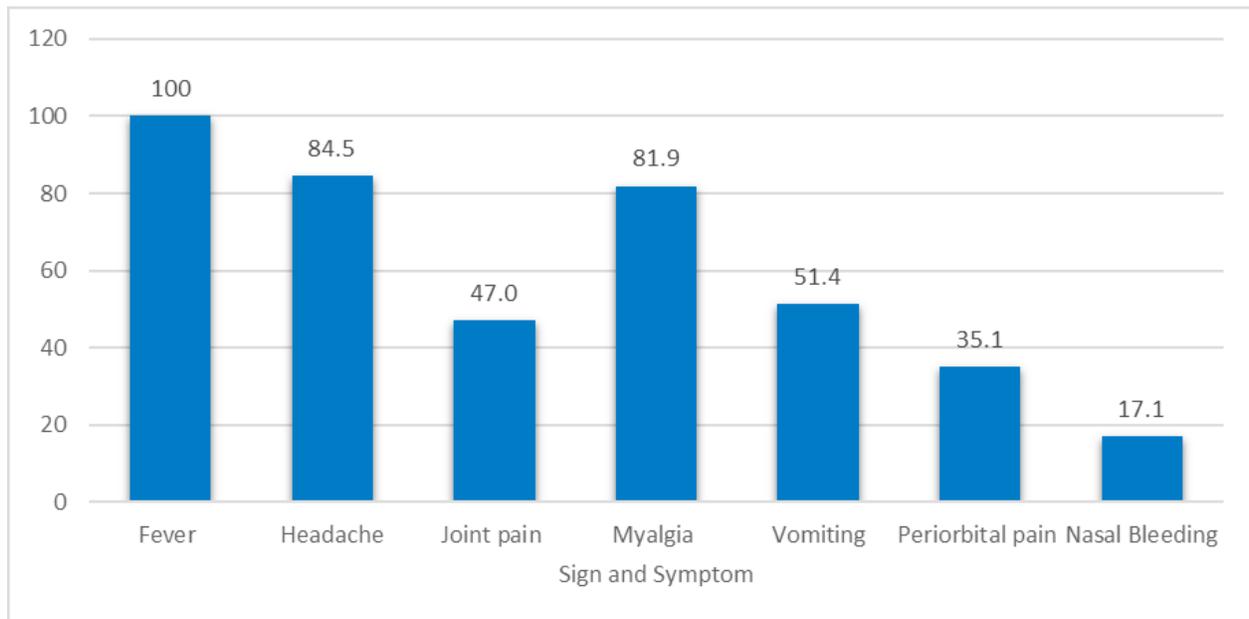


Figure 2: Dengue fever sign and symptoms in Mille Woreda, Afar region May 2023.

Health Institute for analysis. Due to limited resources, only 10 serum samples were tested at the EPHI by RT-PCR. Of the 10 suspected cases, 8 (80%) were seropositive for dengue virus serotype 3. The rest of the cases were epidemiologically linked by person, place, and time.

Environmental Investigation

A total of 207 homes were included in the entomological investigation from April 14, 2023, to May 7, 2023. The team examined 243 water containers, and the majority (62%) of cases and controls did not have proper waste disposal. Of the 207 homes examined, mosquitoes were found at 22 potential breeding sites or water-holding containers. Several mosquito breeding sites were examined and identified in the study area, including discarded

tires, plastic bottles, Jericans, and surrounding man-made stagnant water, which were common in the affected communities. Owing to the hot weather in the town, most people wear short-sleeved shirts and sleep outside during the day. *Aedes aegypti* and *Aedes africanus* mosquito species were identified in the study area using the standard morphological identification key developed by Rueda [15-18].

Case-Control Study

Socio-Demographic Characteristics

We interviewed 57 patients (median age, 31 years; IQR, 22 years) and 114 controls (median age, 35 years; IQR, 19 years). Of all the respondents, 103 (60%) were males (Table 2). Of the total cases,

36 (63.2%) were males and of all the total controls, 67 (59%) were males. The mean age of the patients was 31 years, with a standard deviation (SD) of 15 years, and the control group was 39 years, with an SD of 13 years. Of the total cases, 20 (35%) and 37 (65%) were single and married, respectively, and of the total controls, 35 (31%) and 79 (69%) were single and married, respectively. Of the total cases, 31 (54%) were jobless, while of the total control, those employed in the private sector and government employees were 23 (40%) and 3 (6%), respectively. A total of 31 (54%) had no formal education. However, of all the total controls, 52 (46%) had formal education (Table 2).

Table 2: Demographic Characteristics of Dengue Fever Cases and Controls.

Variables	Category	Cases N=57	Controls N=114	Total N=171
Sex	Female	21(36.8%)	47(41%)	68 (40%)
	Male	36(63.2%)	67(59%)	103 (60%)
Age group	5-14 years	3(5%)	5(4%)	8 (4.6%)
	15-44 years	47(82%)	95(83%)	142 (83%)
	45and above	7(13%)	14(12%)	21 (12%)
Marital status	Single	20(35%)	35(31%)	55 (32%)
	Married	37(65%)	79(69%)	116 (68%)
Occupation	Jobless	31(54%)	61(53%)	92 (54%)
	Private	23(40%)	45(39%)	68 (40%)
	Governmental	3(6%)	8(7%)	11 (6.4%)
Education	Non-formal	31(54%)	62(54%)	93 (54%)
	formal	26(46%)	52(46%)	78 (45.6%)

Assessment of Knowledge Status Towards Dengue Fever

A total of 28 (49%) cases had heard of dengue fever, while 108 (63%) of the total controls had heard of DF (Table 3). Nearly, 19 (33%) of the cases and 27 (24%) of the controls had no idea of the cause of dengue fever. On the other hand, 15 (26%) of the cases and 71 (62%) of the controls knew the mode of transmission. In addition, 31 (54%) cases were unaware of the symptoms of DF, while 52 (46%) of the total controls were unaware of the

symptoms. Furthermore, 27 (47%) of the total cases and 61 (53%) of controls did not know when mosquito bites occurred. In addition, respondents were asked about the mosquito's need to use water for breeding and the result showed that 18 (33%) of cases and 40 (35%) of controls did not know (Table 3).

Assessment of Associated Risk Factors for Dengue Fever

In all cases, only 2 (4%) had ever been exposed to dengue fever. Nearly, 15 (26%) of all cases and 79 (69%) of controls used LLINs during sleep (Table 4). Availability and use of LLINs were assessed and found that 17 (30%) of the total cases had used LLINs, while 73 (64%) of total controls had accessed to LLINs. Nearly 33 (58%) of respondents from cases and 70 (62%) respondents from controls had open water containers in and around their homes. 7 (12%) of cases and 28 (25%) of control identified the presence of larvae. In addition, 25 (44%) of cases and 37 (33%) of controls were exposed to stagnant water. In addition, 40 (70%) of case respondents and 94 (82%) of control respondents had not sprayed at their homes in the past six months. About 40 (70%) of the cases had close contact with the same complaint, while 83 (73%) of controls had a similar contact. Nearly 2 (4%) of the cases used repellent on the skins, whereas, 5 (4%) of the controls used a repellent. Approximately 5 (9%) of cases and 8 (7%) of the control groups had travelled history of dengue fever-affected areas. In addition, 44 (77%) of cases and 32 (28%) of controls were worn short-sleeved clothing (Table 3).

The bivariable analysis showed strong evidence that not hearing of DF (P:0.008), not knowing the mode of transmission (P:0.025), not having of LLINs (P:0.021), not using of LLINs (P:0.000), and wearing of long-sleeved cloth (P:0.000) were found to be significant risk factors for DF infections and a variable with $p < 0.05$ in bivariate analysis were included in the multivariate logistic regression model (Table 4).

Table 3: Knowledge Towards on Dengue Fever Cases and Controls.

Variables	Category	Cases N=57	Controls N=114	Total N=171	COR (95% CI)	P-Value
Heard about DF	No	29(51%)	34(30%)	63 (37%)	2.43(1.26-4.6)	0.008
	Yes	28(49%)	80(70%)	108(63%)		
Knew symptoms of DF	No	31(54%)	52(46%)	68(40%)	1.37(0.72-2.59)	0.33
	Yes	26(46%)	62(54%)	103(60%)		
Mode of transmission	No	42(74%)	43(38%)	85(49.7%)	4.62(2.29-9.31)	0.00
	Yes	15(26%)	71(62%)	86(50.3%)		
Is DF contagious	No	37(65%)	69(60%)	106(62%)	1.2 (0.62-2.33)	0.57
	Yes	20(35%)	45(40%)	65(38%)		
Time of mosquito bite	No	27(47%)	61(53%)	88((51%)	1.27(0.67-2.4)	0.44
	Yes	30(52%)	53(47%)	83(49%)		
Cause of DF	No	19(33%)	27(24%)	46(27%)	1.61(0.8-3.24)	0.18
	Yes	38(67%)	87(76%)	125(73%)		
Water required for mosquito breeding	No	38(67%)	74(65%)	112(65%)	1.17(0.59-2.3)	0.64
	Yes	18(33%)	40(35%)	58(35%)		

Table 4: Risk Factors Towards Dengue Fever Cases and Controls.

Variables	Category	Cases N=57	Controls N=114	COR (95% CI)	P-Value
Infected previously	No	55(96%)	109(96%)	1.26(0.23-6.71)	0.78
	Yes	2(4%)	5(4%)		
Availability of LLINs	No	40(70%)	41(36%)	4.18(2.11-8.3)	0.000
	Yes	17(30%)	73(64%)		
Utilization of LLINs	No	42(74%)	35(31%)	6.32(3.1-12.8)	0.000
	Yes	15(26%)	79(69%)		
Larvae identified	No	50(88%)	86(75%)	.32(0.94-5.7)	0.06
	Yes	7(12%)	28(25%)		
Available of stagnant water	No	32(56%)	77(67%)	1.62(0.84-3.12)	0.14
	Yes	25(44%)	37(33%)		
Is the water container open?	No	24(42%)	44(38%)	1.15(0.6-2.21)	0.65
	Yes	33(58%)	70(62%)		
House sprayed in the last six months	No	40(70%)	94(82%)	1.99(0.94-4.2)	0.69
	Yes	17(30%)	20(8%)		
Available of river around Village	No	44(77%)	88(77%)	(0.46-2.1)	1
	Yes	13(23%)	26(23%)		
Close contact in the last 2 weeks	No	17(30%)	31(27%)	(0.46-2.13)	0.71
	Yes	40(70%)	83(73%)		
Travel history DF affected area	No	52(91%)	106(93%)	1.27(0.39-4)	0.68
	Yes	5(9%)	8(7%)		
Utilization of mosquito repellent in the skin	No	55(96%)	109(96%)	1.26(0.23-6.71)	0.78
	Yes	2(4%)	5(4%)		
Type of clothes wear during daytime	Short sleeves	44(77%)	32(28%)	.115(0.05-0.24)	0.000
	Long sleeves	13(23%)	82(72%)		

Table 5: Bivariate and Multivariate Analysis for factors Associated with Dengue Fever Outbreak.

Characteristics	Cases n=57	Controls n= 114	Bivariable analysis		Multivariable analysis	
			COR (95% CI)	P-Value	AOR (95%CI)	P-Value
Heard about DF						
No	29(51%)	34(30%)	2.43(1.26-4.6)	0.008	3.76(1.46-65)	0.006
Yes	28(49%)	80(70%)				
Mode of transmission						
No	42(74%)	43(38%)	4.62(2.29-9.31)	0.000	2.88(1.14-7.25)	0.025
Yes	15(26%)	71(62%)				
Availability of LLINs						
No	40(70%)	41(36%)	4.18(2.11-8.3)	0.000	2.85(.56-6.97)	0.21
Yes	17(30%)	73(64%)				
Utilization of LLINs while sleeping						
No	42(74%)	35(31%)	6.32(3.1-12.8)	0.000	6.83(2.63-17.7)	0.000
Yes	15(26%)	79(69%)				
Type of cloths wear during day time						
Short Sleeved	44(77%)	32(28%)	.115(0.05-0.24)	0.000	.11(.04-.28)	0.000
Long sleeved	13(23%)	82(72%)				

In multivariate logistic regression, we found that those who had not heard of DF (AOR: 3.76, 95% CI: 1.46–9.65), did not know mode transmission (AOR: 2.88, 95% CI: 1.14–7.25), not using LLINs (AOR: 2.85, CI: 1.16–6.97) and Utilization of LLINs (AOR: 6.83, 95% CI: 2.63–17.7) were independent risk factors for DF. Whereas, wearing long-sleeved (AOR: 0.11, 95% CI: 0.04–0.28) was protected against contracting DF (Table 5).

Public Health Actions

In the affected study area, community sensitization, social mobilization, multidisciplinary coordination and collaboration, chemical spraying, and communication were conducted to prevent

and control mosquito breeding sites. In addition, communities were encouraged to undertake active case searches and enhance activities to prevent and control mosquito breeding sites such as drainage of water bodies.

Discussion

The current investigation showed that Dengue Fever in Mille woreda males were more affected than female residents This finding is consistent with a study conducted in Bihar, Eastern India [19], and Zhejiang, China [20]. This observation might be related to that males spent most of their time outside of the house and due to the nature of the *Aedes aegypti* mosquito, which is

active and bites human beings during the day time cause males wearing short-sleeved clothes [21]. However, the study conducted in North-East Brazil indicated that females (65%) were more affected than Males [22].

The current study indicated that all DF patients in Mille Woreda had 100% fever. This is consistent with the investigation conducted at werder woreda, dollo zone Somali, Ethiopia [23], University of Malaya Medical Centre Kuala Lumpur, Malaysia [24].

In our study, the overall attack rate was 367 per 10,000 which is higher than the studies conducted in the Kabridahar District, Somali Region, Ethiopia [25], and rural areas of Islamabad, and Pakistan [26]. This difference might be due to the poor implementation of prevention and control strategies in the study area. In our findings, the age-specific attack rate in the 15–44 age groups were 477.5 per 100,000. This attack rate is lower than in a study conducted in Asyaita and Dupti districts, Afar Region. This might be because the age group 15–44 years of residents in the latter study used long sleeved clothing.

Mille woreda residents' awareness of DF was assessed and as a result, 63% of participants had heard of DF, of which 28/49% were cases. However, in a study conducted in Dire Dawa, only 40% of participants had heard of DF [27]. This difference might be due to the recurrent occurrence of DF in Mille woreda. 51% of cases had no idea of the symptoms of DF, while the rest of the cases had a considerably good awareness of DF symptoms. This result is in contrast with a study conducted in Gujrat, Pakistan [28]. This difference may be due to a lack of dengue awareness campaigns in the study area.

Thirty-three percent of cases had an idea that water was required for mosquitoes to breed. However, the study conducted in Dire Dawa [27] showed that nearly a quarter of the patients knew stagnant water was a favorable breeding site. This difference may be due to people who have been exposed to a previous outbreak of DF. The current study showed that people who have not heard of DF were 3.8 times more likely to be exposed to DF than those who have heard of it. This finding contrast with the study conducted in the Pwani Region of Tanzania [29].

The present study showed an association between DF and knowledge of the mode of DF transmission; those people who do not know about the mode of DF transmission were 2.9 times more likely to be affected by DF than those who know DF transmission. This finding is consistent with the study conducted in Malaysia [30]. The present study showed a significant association between DF and LLIN use, showing that people who have not used LLINS were 6.8 times more likely to be exposed to DF infection than those who use it. Similar findings were reported by other studies conducted in Dire Dawa, Ethiopia [27], There is also similar study conducted in Luanda, Angola also showed that having used a bed net in the past 30 days was significantly associated with protection from recent Dengue infection ($p = 0.05$) [31]. this might be due to the unavailability and distribution of mosquito bed nets. However, the study conducted in Lahore, Pakistan showed that, there was no

association was found between dengue infection and people not using nets during sleeping [32].

The current study showed that wearing long-sleeved was found to be a protective factor against DF infection, with a nearly 89% reduction in infection. This finding agrees with the study conducted in rural areas of Islamabad, Pakistan [26].

Conclusion

Our epidemiological, entomological, and laboratory investigation confirmed a DF outbreak in Mille Woreda. In this study males were more affected than female residents of Mille woreda and the highest proportion of cases were among the age group 15-44 years old. Not hearing about DF, not knowing the mode of transmission, not using Long Lasting Insecticide Nets, and not spraying insecticide were independent risk factors for the occurrence of dengue fever whereas wearing long-sleeved was an independent protective factor.

- In the long term, we recommend improved vector surveillance and control programs
- Promoting best practices in preserving water
- Disposal of containers in reducing Aedes density.
- Similar recommendations have been made as a result of previous DF investigations within Ethiopia [33,34] as well as in a continent-wide systematic review of DENV infection [35],
- We echo these to prevent and manage future outbreaks.
- Additionally, healthcare worker education is recommended, which is also supported by findings from this same continent-wide systematic review [35].

This approach may lead to earlier identification of outbreaks, better case management, additional sample collection, capacitating regional laboratories and strengthened surveillance system should be essential methods to control future outbreaks

Declarations

Ethics approval and consent to participate

Ethical clearance was obtained from the Institution Review Board (IRB) of the Ethiopian Public Health Institute. Official permission was also obtained from Mille woreda health office, Zone One, Afar Region. Written informed consent was obtained from the study participants. For those aged, less than 18 years, informed written consent was obtained from respective parents or guardians and it was performed in accordance with the Declaration of Helsinki. The individual results of any investigation remained confidential.

Consent for Publication

Support letters were written to the Afar Regional Health Bureau to obtain permission and to facilitate the investigation process. Serum samples were collected only aiming to investigate the causative agent of the unusual febrile illness and to guide appropriate outbreak control interventions. Verbal informed consent was obtained from all identified cases that were greater than 18 years of age. Confidentiality of patient information was kept by using code number and accessed by only the principal and co-investigators.

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