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Personal and environmental risk factors for chikungunya virus infection: cross-sectional hospital-based study among febrile patients receiving treatment in AI-Hodayda city hospitals, Yemen

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ABSTRACT

Background: Mosquitoes carry the illness known as chikungunya, which is becoming more common throughout Yemen. It is a serious matter concerning public health. In the hospitals located in AI-Thawrah, AI-Amal, and AI-Aqsa in AI-Hodayda city, Yemen, feverish patients seeking treatment were the subjects of this study, which aimed to investigate host and environmental risk factors.

Methods: Patients with fever were enrolled in the trial at the authorized hospitals in AI-Hodayda city between June and June 2023. For febrile patients, a set of questionnaires was employed to collect clinical, sociodemographic, and related risk factors. Using an enzyme-linked immunosorbent assay, patients were tested for chikungunya antibodies as well as immunoglobulin M (IgM) and G (IgG). Reverse transcriptase polymerase chain reaction (RT-PCR), which searches for CHIK viral RNA, was used to confirm the findings.

Results: In all, 120 patients with fever were included in the research. According to RT-PCR, ELISA, and/or RDTs, the total prevalence of chikungunya infection was 14 (11.7%). The study found a correlation between gender and chikungunya infection, with males having a slightly higher rate (12.2%) compared to females (10.9%). The age group of 22-31 years had a higher rate (15.4%) than the ≤21 (7.3%) and a slightly higher (12.5%) group of ≥32 years. The study also identified risk factors associated with chikungunya infections in individuals experiencing fever in Hodiedah city. These included living in a rural area, living in a bog, lacking sewage in homes, failing to use a bed net, having an exposed water tank, not having a window screen, not wearing full body cloths, having trash around or near the patients' houses, and having another ill person with a fever of unknown origin in the patients' houses.

Conclusions: The research involved 120 patients with fever, revealing a 11.7% prevalence of chikungunya infection. Gender was found to be a significant factor, with males having a slightly higher rate. Age groups 22-31 had a higher rate than other age groups. Risk factors associated with chikungunya infections included rural living, bog living, lack of sewage, bed net use, exposed water tanks, and trash.

CONTENTS

1. Introduction:

Chikungunya can infect humans when an Aedes mosquito bites them. The Chikungunya virus (CHIKV) is mostly transmitted by Aedes aegypti and Aedes albopictus, although it is also sustained in sylvatic cycles involving monkeys and Aedes species present in forests [1]. Chikungunya is a global public health concern because of its continued growth and evolving epidemic patterns throughout the world's tropical and subtropical regions, particularly in Yemen [2-4]. This is connected to global travel, uncontrolled urbanization, unplanned urbanization, and climate change [1]. Since the 1960s, CHIKV has been placed under isolation several times throughout Africa, Asia, and Latin America. In 25 African countries, there have been reports of chikungunya epidemics [5].

In the past ten years, there have been two significant outbreaks of chikungunya in the coastal regions of southwest Yemen [2, 3]. Chikungunya and other widely dispersed febrile illnesses such as brucellosis [6], TB [7], leptospirosis [8], vessiral leishmaniosis [9, 10], typhoid [11], and toxoplasmosis [12] can coexist in Yemen. Yemen, like many other tropical and subtropical countries, is experiencing a change in the causes of febrile illnesses. While the number of cases of non-malarial febrile illnesses is increasing, the incidence of malaria-related febrile illnesses is decreasing in the Middle East [13]. Raboviruses are being more recognized as the most likely cause of some non-malarial febrile syndromes, as demonstrated by an increase in viral disease outbreaks transmitted by mosquitoes [14].
Recent studies carried out in Yemen have revealed that patients with acute dengue and chikungunya infections are often misdiagnosed and treated with antibiotics or antimalarials, primarily due to a lack of differential diagnosis [15]. The majority of Yemeni healthcare facilities do not regularly check for the causes of fever cases that coexist with malaria. 17% of all infectious illnesses affecting humans worldwide are caused by viruses carried by arthropods [16, 17]. Mosquito-borne diseases are the most significant type of arboviral infections, impacting millions of individuals and accounting for a significant share of newly and re-emerging human pathogens. It has been reported that diseases like dengue, yellow fever, Japanese encephalitis, chikungunya, and Rift Valley fever significantly affect disability-adjusted life years [18, 19, 20]. Arboviral infections are spreading more widely due to a number of factors, including international travel, unplanned settlements, growing human population worldwide, and changing and unpredictable environments [21–24]. Because of cycles including human-human and human-peri-domestic Aedes mosquito transmission, this raises the risk of viral infections spread by mosquitoes [24, 25].
Although there is proof that arboviral illnesses like dengue and chikungunya significantly increase morbidity in Yemen, the burden, causes, and susceptibility of these infections have only been partially studied. This implies that it is yet unclear where they will be found throughout the nation. Determining the seroprevalence and risk factors of chikungunya in Hodiadah city was, thus, the aim of this study.

2. Subjects And Methods

Study design: This study is a cross-sectional study.

Study subjects and study area: All patients with severe fever (≥ 38°C) who are admitted to

the emergency room at AI-Thawrah, Al-Amal, and Al-Aqsa hospitals in AI-Hodayda city and who show two or more of the following symptoms—headache, arthralgia, myalgia, and skin rash—are considered selected cases.

Exclusion criteria: Patients who are already diagnosed with other diseases such as enteric fever or non-febrile illness were excluded.

Sample size: The following information was used by the Epi Info statistical program version 16 (CDC, Atlanta, USA) to statistically compute the sample size: The Central Statistical Office states that 415,283 people make up the study's population. In a prior study, the illness prevalence was 5.5% [3]. A 95% confidence level is needed with a 5% allowable margin of error. Eighty febrile patients were expected to be in the study sample. The sample size was expanded to 120 febrile individuals in order to improve accuracy.

Data collection: A pre-made questionnaire was used to gather patient information, which included demographic information, clinical signs and symptoms, potential risk factors, and test findings.

Specimen collection: In order to collect the desired sample, approximately 5 milliliters of venous blood were extracted under sterile conditions from feverish patients (during the first week of fever) and placed into two distinct sterile bottles; the first bottle, which contained anticoagulant, held one-third of the total blood and was utilized for rapid tests for malaria and CBC, while about 20 milliliters of the blood were used to prepare thin and thick blood films for malaria research. Of the total blood that was allowed to clot at room temperature, two-thirds was contained in the second bottle, which was anticoagulant-free. The serum was divided into two distinct Eppendorf tubes, one of which would serve as the primary sample and the other as a backup, and it was then kept at -20 °C until it was needed for PCR molecular analysis.

The same febrile patient had approximately 3 milliliters of venous blood drawn during the second week of the infection. The blood was then placed into a sterile (anticoagulant-free) bottle and allowed to clot at room temperature. Both the ELISA and the rabid chikungunya test employed the serum.

1. **Investigation of Chikv**
2. **Molecular Analysis Of Chickv**
3. **RNA extraction:** The genomic viral RNA was extracted from the serum of each patient according to the manufacturer's instructions using a CHIKV extraction kit obtained from Qiagen. Chikungunya RT-PCR Kit (Altona Diagnostics, Hamburg, Germany) using 200µl of serum or plasma.

2. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) for detection of CHIK viral RNA:

CHIKV RNA was quantified and detected by the determination kit for Chikungunya virus RNA (HELINI Biomolecules, India) using reverse transcription polymerase chain reaction, according to the manufacturer's instructions.

B. Serology

1. **Chikungunya-specific IgM enzyme-linked immunosorbent assay (ELISA):** All serum specimens were screened for CHIKV-specific IgM antibodies by ELISA using a commercial kit (InBios International, USA) according to the manufacturer's recommended procedure.

2. **Rapid diagnostic tests for CHIKV IgM detection (RDT):** All serum specimens were screened for CHIKV-specific IgM and IgG antibodies using the Chikungunya IgM rapid test kit (SD BIOSENSOR, Germany) according to the manufacturer's instructions.

Statistical Analysis: Version 7 of Epi-Info Statistics was utilized to evaluate the information. The associated odds ratio, 95% confidence interval, chi square, and p values for the risk factors linked with the prevention of

Chikungunya virus infections were ascertained using statistical analysis.

Ethical Consideration: Contract No. 317 was given ethical approval on January 1, 2022, by the Medical Ethics and Research Committee of Sana'a University's Faculty of Dentistry. The review committee's established ethical guidelines were constantly adhered to. The selected individuals gave their written and informed consent.

3. Results

This cross-sectional study, included 120 patients with general symptoms suspected to had chikungunya concurrent with malaria in Hodeida city. The results which were obtained from the present study were analyzed, documented, and presented in 4 tables. The features of febrile patients suspected of having chikungunya who were enrolled in this study are described in Table 1.

Table 1: Sociodemographic characteristic of febrile patients attending the hospitals of Hodeidah city- Yemen.

Characteristics	**Patients n=120**	
	No.	%
Gender		
Male	74	61.7
Female	46	38.3
Age groups (yrs.)		
≤21	41	34.2
22-31	39	32.5
≥32	40	33.3
Median (IQR)	27.0(18.0-35.0)	
Marital status		
Single	69	57.5
Married	51	42.5
Residence		
Urban	104	
Rural	16	13.3
Education status		
Illiterate	08	6.7
Primary	22	18.3
Secondary	59	49.2
University	31	25.8

In terms of gender, 46 patients (38.3%) were female and 74 patients (61.7%) were male. The age range covered eight to seventy years, with a median age of twenty-seven (18.0-35.0). Regarding place of residence, 104 participants (86.7%) lived in urban regions, while 16 participants (13.3%) lived in rural areas. Lastly, concerning educational status, nearly half of the study's 59 participants (49.2%) had completed secondary school. In AI-Hodayda **city**, Yemen, Table 2 displays the correlation between gender and chikungunya infection.

Table 2: The association between gender and chikungunya infection in Hodeida city-Yemen.

Gender	Chikungunya				Total **(n=120)**		*OR*	*CI*	X^2	*P*
	+ve **(n=14)**		-ve **(n=106)**							
	No.	%				%				
Male(n=74)	9	12.2	65	87.8	74	61.7	1.1	0.3-3.6	0.04	0.8
Female(n=46)	5	10.9	41	89.1	46	38.3	0.1	0.2-3.6	0.04	0.8
Total	14	11.7	106	88.3	120	100				

OR Odd ratio > 1 at risk CI =Confidence intervals 95%
χ^2 =Chi-square ≥ 3.84 *p* = Probability value

The rate of chikungunya infection in males was somewhat higher (12.2%) with OR = 1.1 compared to the rate in females (10.9%) with OR = 0.1; however, this difference was not statistically significant. The relationship between age and chikungunya infections in AI-Hodayda **city,** Yemen, is displayed in Table 3.

Table 3: The association between age and chikungunya infections in Hodeida city- Yemen.

Age groups	Chikungunya				Total (n=120)		*OR*	*CI*	X^2	*P*
	+ve (n=14)		**-ve (n=106)**							
	No.	**%**	**No.**	**%**						
≤21 **(n=41)**	3	7.3	38	92.7	41	34.2	**0.4**	**0.1-1.8**	**1.1**	**0.28**
22-31 **(n=39)**	6	15.4	33	84.6	39	32.5	1.6	0.5-5.1	0.7	0.37
≥32 **(n=40)**	5	12.5	35	87.5	40	33.3	1.1	0.35-3.6	0.04	0.84
Total	**14**	**11.7**	**106**	**88.3**	**120**	**100**				

χ^2 Chi-square ≥ 3.84
p Probability value ≤ 0.05 (**significant**)

The age group of 22-31 years had a higher (15.4%) OR = 1.6 rate of chikungunya infection than the ≤21 (7.3%) OR = 0.41 group, and a slightly higher (12.5%) OR = 1.1 group of ≥32 years; however, the differences were not statistically significant. Table 4 delineated the plausible risk factors associated with chikungunya infections in individuals experiencing fever in Hodiedah city. Living in a rural area was associated with a higher risk of contracting chikungunya infection (OR=25 times, 95% CI=6.6-96, X2=35, p<0.0001). Bogs and the incidence of chikungunya infection were associated in a way that was equal to OR=2.2 times with 95% CI equal to 0.6 – 6.5, X^2 equal to 1.7, and p=0.18 (not a statistically significant finding). A correlation was seen between the patients' lack of sewage in their homes and the incidence of chikungunya infection, as indicated by OR=2.1 times with 95% CI = 0.7 – 6.5, X^2 = 1.7, and p = 0.18 (not statistically significant result). The patients' failure to utilize a bed net was associated with an OR of 8.1 times with a 95% confidence interval of 0.5 to 137 cases of chikungunya infection, X^2 of 2.9, and p of 0.08 (not statistically significant result). An link was found between the presence of an exposed water tank in the patients' home and the incidence of chikungunya infection, with an OR of 14.1 times, 95% confidence interval (CI) of 2.1 to 94, X^2 of 11.8, and p<0.0001. The absence of a window screen in the patients' homes was associated with an increased risk of chikungunya infection, corresponding to OR= 8.1 times with 95% CI = 0.5 – 137, X^2 = 2.9, and p = 0.08 (not statistically significant finding).There was an association between no full body cloths of the patients and the occurrence of chikungunya infection equal to OR = 8.1 times with a 95% CI equal to 0.51–137, with X^2 = 2.9 and p = 0.08 (not a statistically significant result). There was an association between the presence of trash around or near the houses of the patients and the occurrence of chikungunya infection equal to OR = 5.8 times with a 95% CI equal to 1.2 - 27, with X^2 equal to 6.1 and p = 0.01. There was an association between the presence of another ill person with a fever of unknown origin in the houses of the patients and the occurrence of chikungunya infection equal to OR = 4.3 times with a 95% CI equal to 1.4 - 14, with X^2 equal to 6.8 and p = 0.008.

Table 4: Associated risk factors of contracting chikungunya infection in Hodiedah city-Yemen.

Risk factor	**Chikungunya**		**Total (n=120)**	OR	CI	X^2	p
	+ve **(n=14)**	-ve **(n=106)**					

		No.	%	No.	%	No.	%				
Residence	Urban	05	35.7	99	82.5	104	86.7	0.03	0.01-0.14	35	<0.0001
	Rural	09	64.3	07	5.8	16	13.3	25	6.6-96	35	<0.0001
Blood transfusion	Yes	0	0	1	0.83	1	0.8	0	undefined	0.13	0.7
	No	14	100	105	87.5	119	99.2	Reference			
Presence bogs	Yes	05	35.7	21	17.5	26	21.7	2.2	0.6-6.5	1.7	0.18
	No	09	64.3	85	70.8	94	78.3	Reference			
Presence sewages	Yes	08	57.1	41	34.2	49	40.8	Reference			
	No	06	42.9	65	54.1	71	59.2	2.1	0.7-6.5	1.7	0.18
Bed net use	Yes	0	0	0	5.8	07	5.8	Reference			
	No	14	100	100	82.5	113	94.2	8.1	0.5-137	2.9	0.08
Un-Covered water tank	Yes	11	78.6	104	86.7	115	95.8	14.1	2.1-94	11.8	<0.0001
	No	3	21.4	02	1.7	05	4.2	Reference			
No Windows screen	Yes	14	100	80	66.7	94	78.3	8.1	0.5-137	2.9	0.08
	No	0	0	26	21.7	26	21.7	Reference			
Full body cloths	Yes	0	0	07	5.8	07	5.8	Reference			
	No	14	100	99	82.5	113	94.2	8.1	0.5-137	2.9	0.08
Presence of trash	Yes	12	85.7	54	45	66	55	5.8	1.2-27	6.1	0.013
	No	2	14.3	52	42.5	53	44.2	Reference			
Presence of ill person in the house hold	Yes	9	64.3	31	25.8	40	33.3	4.3	1.4-14	6.8	0.008
	No	5	35.7	78	65	80	66.7				

OR Odd ratio > 1 at risk CI =Confidence intervals 95%
χ^2 =Chi-square ≥ 3.84 *p* = Probability value

4. Discussion

This is believed to be the first cross-sectional study on chikungunya in feverish patients conducted in Yemen in general and in AI-Hodayda city specifically. Although AI-Hodayda **city** has been identified as one of the Chikungunya endemic regions [26], no efforts have been taken to carry out epidemiological investigations about the matter. Fourteen individuals (or 11.7%) of the study's subjects had chikungunya infections. Our results corroborated recent reports of 28% CHIKV seroprevalence among feverish patients in Yemen [2]. Furthermore, they were lower than those reported in Sudan (73.1%), Zambia (36.9%) [27], Cameroon (51.4%), Nigeria (15%), and Zambia [28]. However, compared to Rezza et al. (1988), our results were higher in AI-Hodayda city (9.8%), Hadramout (0% [30], Djibouti (2.6% [31], Iraq (0.5% [32], Senegal 0.02% [33], Tanzania (0.6% [34], and Eastern and Central Sudan 1.8% [35].

Males had a slightly higher (12.2%) OR = 1.1 chikungunya infection rate than females (10.9%) OR = 0.1; however, this difference was not statistically significant. Male sex was more likely than female sex to be infected with CHIKV, and several studies from Malaysia [36], Nigeria [37], Bangladesh [38], and India [39] have connected male sex to an increased risk of CHIKV and concurrent infection.

The cultural norms and practices that render men more susceptible to Aedes species assaults could account for this gender prejudice. Men are also more courageous; they hunt and support their families no matter where they are or when. Male practices in hot climates, like exposing their arms and legs with cut garments, are thought to increase the risk of infection compared to females who dress modestly and go outside unnoticed. This observation aligns with earlier Yemeni research projects [40, 41]. Our results, however, conflict with earlier findings that

women were more likely than men to be affected by the CHIFV mono-infection, such as those from Southwest Nigeria [45], Reunion Island [43, 44], and Colombia [42].

Table 3 shows the association between chikungunya infections and age in AI-Hodayda city, Yemen. While the differences were not statistically significant, the age group of 22–31 years had a higher (15.4%) OR = 1.6 rate of chikungunya infection than the ≤21 (7.3%) OR = 0.41 group and a slightly higher (12.5%) OR = 1.1 group of ≥32 years. Our results are comparable to the study results from Tanzania, where the majority of CHIFV infections were in the second and third decayed years of life [46]. One explanation for this could be that the participants, whose ages vary from 22 to 31, are actively employed and spend more time outside engaging in various jobs or leisure pursuits. The bulk of patients with CHIKV infections in the current investigation were adults in general. Other investigations have documented the arboviruses' propensity to impact older populations. The adult age groups had the highest prevalence of recent CHIFV infections, according to a recent study conducted in Vietnam [47]. In a similar vein, elderly people had greater rates of CHIFV infection prevalence than younger people, which is consistent with research conducted in Malaysia [48] and other African countries [49]. There has also been recent reporting of low CHIFV infection prevalence in people under the age of 15 in Cameroon [50]. Similar to the present investigation, the age correlation with CHIFV infection in Zambia showed that those under 5 years of age had a lower probability of being seropositive for the virus than those over 45 years of age [51]. It was highest in people between the ages of 20 and 45 in a Brazilian study [52]. Table 4 listed the potential risk factors for chikungunya infections in AI-Hodayda city residents who were feverish. An increased risk of chikungunya infection was linked to living in a rural region (OR=25 times, 95% CI=6.6-96, X^2=35, p<0.0001). People living in rural areas were notably more vulnerable to CHIKV circulation than people living in urban areas. On the other hand, no correlations between the study site and arbovirus co-circulation were found in a Malaysian investigation [48].

5. Conclusion

In conclusion, the results of this study provide evidence of the spread of CHIFV in diverse environmental regions in Yemen. The widespread distribution of CHIKV infection in the country underscores the need for more effective surveillance to monitor the incidence and spread of CHIFV infection. It is important that surveillance and diagnostic systems for CHIFV infection be strengthened nationally and that information on other arboviruses be captured. The prevalence and similarity in modes of transmission of arboviruses observed in Yemen call for steps to improve the differential diagnosis of febrile syndromes in order to improve management and clinical outcomes.

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Data Availability

The data will be available to anyone upon request from the corresponding author.

A Dispute Of Interest

Regarding this project, there is no conflict of interest.

Author's Contributions

Nagwa Abdo Ali Mohammed did the fieldwork for this study as part of a PhD in the department of Medical Microbiology, Faculty of Medicine and Health Sciences, Sana'a university. Other authors including prof Hassan al-Shamahy assisted with data analysis, drafting and reviewing the manuscript, and giving final clearance to the study.

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