A Comprehensive Seroepidemiology of Dengue and Chikungunya Arboviruses in Iran, 2020-2023

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Abstract

Aim: are the most common arthropod-borne viruses (Arboviruses) globally. So far, no autochthonous cases of DENV and CHIKV infections have been reported in Iran. Nevertheless, due to the recent identification of *Aedes aegypti* in the south and *Aedes albopictus*in the north of Iran, the country is at a serious risk of local outbreaks of these infections.

Methods: Between 2020 and 2023, a comprehensive cross-sectional study was conducted to explore the anti-DENV and CHIKV IgG antibodies in the general populations of both southern and northern regions of Iran using ELISA.

Results: A total of 11192 participants were included, of whom 2.3% (256 individuals) and 0.1% (11 cases) were DENV seropositive and CHIK seropositive, respectively. Across the eight examined provinces, Sistan and Baluchestan province displayed the highest prevalence of dengue seropositive cases (6.80%) (P<0.001). Samples collected in 2022 demonstrated a notably higher seropositive rate of 5.5% (P<0.001). Interestingly, all 11 CHIKV seropositive cases were from Sistan and Baluchestan province.

Conclusion: The seroprevalence of DENV was notably higher in southern regions, particularly in Sistan and Baluchestan province and CHIKV seropositive cases were exclusively found in Sistan and Baluchestan province. This study furnishes crucial insights into the seroepidemiology of DENV and CHIKV in Iran which, in conjunction with the presence of *Ae. Aegypti* in the south and *Ae. Albopictus* in the north of Iran, emphasizes the requirement of improving integrated surveillance and control systems in the whole of the country, especially in the south of Iran.

1. INTRODUCTION

Arthropod-borne viruses (arboviruses) are a diverse group of viruses transmitted to vertebrate hosts by hematophagous arthropod vectors such as mosquitoes, ticks, sandflies, and biting midges [1–3]. Over 500 arboviruses have been identified, of which 150 species can cause infections in humans [4, 5]. Among all medically important arboviral diseases, dengue and chikungunya are the most common, with an annual incidence of 96 million and 693 thousand cases, respectively [6]. Dengue virus (DENV) is a positive-sense single-stranded RNA virus belonging to the genus Flavivirus in the family Flaviviridae. Dengue virus infection can lead to dengue fever (mild form) or severe dengue (dengue hemorrhagic fever and dengue shock syndrome). Dengue fever occurs after an incubation period of 4–7 days (range 3–14 days), the most common symptoms of which include sudden onset of fever, severe headache, eyeball pain, facial flushing, skin rash, myalgia, and joint pain. Dengue fever is a self-limiting disease and recovery is achieved within a few days. However, infection with dengue virus can cause severe dengue, a fatal disease, with an estimated annual death toll of > 20,000 people [7].

Chikungunya virus (CHIKV) is a positive-sense single-stranded RNA virus classified in the genus Alphavirus of the *Togaviridae* family. The incubation period of chikungunya is, on average, between 3 and

7 days (range 1–12 days). The main characteristics of chikungunya are sudden onset of fever and severe joint pain. Other symptoms and signs include headache, back pain, muscle pain, nausea, vomiting, joint inflammation, rash, and conjunctivitis. The clinical symptoms of the acute form can be mild, moderate, and severe, but mostly recover within three weeks. Between 10 and 15% of cases progress to the subacute or chronic forms [8].

Both dengue and chikungunya viruses, which are transmitted to humans mainly by *Aedes aegypti* and *Aedes albopictus* mosquitoes [4], are mostly limited to tropical and subtropical regions. However, due to climate change, vector adaption, globalization, unplanned urbanization and increased international travel and trade in recent years, their distribution range has expanded to new regions, including Europe, North America, and the Middle East [9, 10].

Historically, *Aedes aegypti* was recorded in the cities of Khorramshahr in 1920 and Bushehr

in the early 1950s [11, 12] with no other report of this species until 2019 when it was initially reported in Bandar-e Lengeh and then in 2020 in Bandar-e Abbas in Hormozgan Province, south of Iran [13, 14]. This species was subsequently reported in 2023 in Bandar-e Chabahar in Sistan and Baluchestan Province, south of Iran. *Aedes albopictus*, on the other hand, was initially reported in 2009 and 2013 in Bandar-e Chabahar [15] although failed to establish there. However, this species invaded Guilan Province and established itself there in 2023 (Azari Hamidian, 3rd International Congress on Vector-Borne Diseases and 5th National on Medical Entomology).

Despite conducting several seroepidemiology studies in Iran, the sample size of these studies was small and generally limited to one region of the country. For example, in 2013, Chinikar et al. reported a DENV seroprevalence rate of 5% in 300 Iranian Crimean–Congo Hemorrhagic Fever Disease (CCHF)-suspected patients [16]. In 2014, Aghaie et al. demonstrated that 5.9% of 540 blood donors of Chabahar city in Sistan and Baluchestan Province were DENV seropositive [17]. In another study, 5.9% of 1275 febrile patients from different provinces of Iran were found to be DENV seropositive [18]. Although numerous outbreaks of dengue and chikungunya have been recorded in the Middle Eastern countries, such as Djibouti, Pakistan, Saudi Arabia, Sudan, and Yemen in recent years [10], no autochthonous cases of these diseases have been identified in Iran. However, the recent invasion and establishment of the *Aedes aegypti* and *Aedes albopictus* mosquitos in the south and north of Iran has raised serious concerns regarding the local outbreaks of dengue and chikungunya in Iran [19].

Therefore, to investigate the risk of transmission of dengue and chikungunya, the present study was designed to comprehensively assess the seroepidemiology of these two diseases in the southern and northern border provinces of Iran, which are vulnerable to invasion and establishment of the Aedes vectors.

2. MATERIALS AND METHODS

2.1 Study area and sample collection

In this cross-sectional study, which was conducted from September 2020 to April 2023, the general population of the southern and northern entry points of Iran, including Hormozgan, Bushehr, Khuzestan, Sistan and Baluchestan, Kerman Province from south of Iran, and Guilan, East Azarbaijan and West-Azarbaijan Provinces from north of Iran, were included (Fig. 1).

After receiving an inform consent and completing the questionnaire, a whole blood sample was obtained from participants who were referred to medical laboratories for a routine check-up and at the time of sampling, they did not have any clinical symptoms suspected of DENV and CHIKV infections. The sera were isolated and sent to the Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory) of the Pasteur Institute of Iran in a cold chain for laboratory analysis.

The Ethics Committee of the Pasteur Institute of Iran approved this research.

2.2 Anti-Dengue Virus/ Anti Chikungunya Virus IgG ELISA

To detect anti-DENV and anti-CHIKV IgG antibodies, serum samples were tested by Anti-Dengue Virus ELISA (IgG) and Anti-Chikungunya Virus ELISA (IgG) kits (Euroimmun, Germany) according to the manufacturer's instructions. Briefly, 100 µl of the diluted serum samples, calibrators, and controls were transferred into the microplate wells and incubated for 30 minutes at room temperature. After washing the microplate (3 times), 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) was transferred into each well, and incubated for 30 minutes at room temperature. Following three wash steps, 100 µl of chromogen/substrate solution was added and after a 15-minute incubation at room temperature, the reaction was stopped with 100 µl of stop solution, and the optical density (OD) was measured at 450 nm and 630 nm (reference wavelength) using an ELISA reader. For both ELISA tests, the samples with an OD/ calibrator OD ratio ≥ 1.1 were considered positive, the samples with an OD/ calibrator OD ratio ≥ 0.8 to < 1.1 were considered undetermined results, and the samples with an OD/ calibrator OD ratio < 0.8 were considered negative.

2.3 Statistical analysis

Statistical analyses were performed using the IBM SPSS software, version 23 (SPSS Inc., USA). Descriptive analysis was performed to describe the quantitative data, including mean ± standard deviation (SD) and frequencies. Chi-square, Fisher's exact and independent t-tests were performed to compare the seroprevalence frequency between the gender, province, city and age. A P-value < 0.05 was considered significant.

3. RESULTS

In this study, 11192 serum samples were obtained from individuals living in Bushehr (n = 322, 2.9%), East-Azarbaijan (n = 494, 4.4%), West-Azarbaijan (n = 126. 1.1%), Khuzestan (n = 2071, 18.5%), Sistan and Baluchestan (n = 2658, 23.7%), Kerman (n = 441, 3.9%), Guilan (n = 125, 1.1%) and Hormozgan Provinces (n = 4955, 44.3%). The mean (± SD) age of the subjects was 35.4 (± 14.6) years (range, 1 to 95), and 53.4% (n = 5974) were female. 10860 (97%) of participants were Iranian and the remaining 332 (3%) were

originally from Afghanistan (n = 325), India (n = 3), Pakistan (n = 3) and Bangladesh (n = 1). Regarding profession, the majority of participants were housewives (n = 4460, 39.8%) and freelancers (n = 3491, 31.2%).

408 cases (3.6%) had a history of abroad travel, of which the main destinations were the UAE (n = 157, 38.5%), Iraq (n = 112, 27.4%), Pakistan (n = 106, 25.9%) and Turkey (n = 17, 4.9%).

The results of DENV IgG ELISA revealed that 256 serum samples (2.3%) were positive for DENV IgG, while CHIKV IgG was tested positive by Anti Chikungunya Virus IgG ELISA test in only 11 serum samples (0.1%). All CHIKV seropositive cases were from Sistan and Baluchistan Province (Table 1a) (P-value < 0.001). However, apart from Guilan and West-Azarbaijan Provinces, DENV IgG seropositivity was observed in all provinces with the highest seroprevalence in Sistan and Baluchestan (181/2658, 6.8%), followed by Khuzestan (32/2071, 1.5%), Bushehr (3/322, 0.9%), Hormozgan (37/4956, 0.7%), Kerman (2/441, 0.5%) and East-Azarbaijan Provinces (1/493, 0.2%) (Table 1a) (P-value < 0.001).

As is demonstrated in Table 1b, in Sistan and Baluchestan Province, 6 out of 7 studied cities showed seropositive cases with the highest DENV IgG seropositivity recorded in Rask (13.5%) and Chabahar (11.2%) followed by Sarbaz (7.5), Konarak (4.4%), Saravan (1.4%) and Mirjaveh (0.5%). In Khuzestan Province, 5 out of 6 studied cities had DENV IgG seropositive cases including Dasht Azadegan (3%) and Khorramshahr (2.5%), Ahvaz (1.4%), Bandar-e Mahshahr (1.3%) and Hendijan (0.06%). In Bushehr Province, only Bushehr city showed DENV IgG seropositive cases (2.2%). In Hormozgan Province, DENV IgG seropositivity was observed in 8 out of 10 cities including Bandar Khamir (1.6%), Jask (1.3%), Rudan (1.1%), Minab (1.0%), Sirik (0.8%), Hajiabad (0.7%), Bandar Lengeh (0.4%) and Bandar Abbas (0.3%). In Kerman Province, Menujan was the only city where seropositive cases (1.8%) were detected.

No statistically significant correlation was observed between DENV IgG seropositivity and gender, as female and male participants showed seroprevalence rates of 2.2% and 2.4%, respectively (P-value = 0.644). However, the CHIKV IgG seropositivity was significantly higher in male cases (0.2%) compared to female participants (0.02%) (P-value = 0.13) (Table 2). Neither in the case of DENV nor in the case of CHIKV was there a significant correlation between the seropositivity and nationality (Table 2). Statistical analysis revealed that there was a significant association between age and DENV IgG seropositivity as the mean age was higher in seropositive cases (41.2 ± 15.3) than in seronegative individuals (35.3 ± 14.5) (P-value < 0.001). Although a similar trend was observed about CHIKV IgG seropositivity and age, the difference was not statistically significant (P-value = 0.245). Similar results were observed in comparing the seropositivity rates among different age groups including < 10, 10–20, 21–30, 31–40, 41–50 and > 50 years. Regarding DENV IgG seroprevalence, most seropositive cases belonged to the 41–50 and > 50 age groups (P-value < 0.001). While all CHIKV-positive cases were > 30 years of age, there was no statistically significant association between CHIKV IgG seroprevalence and age group (P-value = 0.890) (Table 2).

DENV IgG was identified in people with different professions and the highest seropositivity rate was observed in farmers (5.7%), healthcare workers (4.1%) and housewives (2.7%) (P-value = 0.001). CHIKV

IgG, however, was identified only in housewives (0.2%) and self-employed cases (0.03%) (P-value = 0.277) (Table 2).

The DENV IgG seropositive cases were detected only in 2021 (83/7559, 1.1%) and 2022 (173/3057, 5.5%) (P-value < 0.001). All 11 CHIKV IgG-positive cases were identified in 2022 (11/3057, 0.4%) (P-value < 0.001) (Table 2).

Interestingly, all CHIKV IgG positive participants reported no history of travel abroad. No statistically significant association was found between travelling abroad and seropositivity for CHIKV. However, 67 out of 256 (26.17%) DENV IgG positive individuals had a history of travelling, among which 61, 5 and 1 had travelled to Pakistan, Iraq, and the UAE, respectively. DENV seropositivity was notably higher in participants who reported a history of travelling abroad (67/408, 16.4%) than those who had not a history of travelling abroad (249/10784, 2.3%) and the difference was statistically significant (P-value < 0.001) (Table 2).

7 participants showed simultaneous positive results for both DENV IgG and CHIKV IgG. All these cases were from Rask city of Sistan and Baluchestan province.

Table 1. Frequency of DENV IgG and CHIKV IgG seropositivity according to the sampling province (1a) and cities (1b) during 2020-2023 in Iran.

1a

CHIKV IgG			DENV IgG			Province
P-value	No. Positive (%) [95% Conf. Interval]	No. Tested (%)	P value	No. Positive (%) [95% Conf. Interval]	No. Tested	
<0.001	11)0.4% ([0.2 - 0.7%]	2658	<0.001	181 (6.8%) [5.9 -7.8%]	2658	Sistan and Baluchestan
	0 (0.0%) [0.0 - 0.007%]	4955		37)0.7% ([0.05 -1%]	4955	Hormozgan
	0 (0.0%) [0.0 - 0.01%]	2071		32 (1.5%) [1 - 2.1%]	2071	Khuzestan
	0 (0.0%) [0.0 – 1.1%]	322		3 (0.9%) [0.1 - 2.6%]	322	Bushehr
	0 (0.0%) [0.0 – 0.08%]	441		2 (0.5%) [0.05 - 1.6%]	441	Kerman
	0 (0.0%) [0.0 – 0.07%]	494		1 (0.2%) [0.005 - 1.1%]	494	East-Azerbaijan
	0 (0.0%) [0.0 – 2.8%]	126		0 (0.0%) [0.0 - 2.8%]	126	West-Azerbaijan
	0 (0.0%) [0.0 – 2.9%]	125		0 (0.0%) [0.0 - 2.9%]	125	Guilan
	11 (0.1%) [0.05-0.17%]	11192		256 (2.3%) [2.1-2.6%]	11192	Total

1b

			DENV IgG	CHIKV IgG
Province	**City**	**No. Tested**	**No. Positive (%)** [95% Conf. Interval]	**No. Positive (%)** [95% Conf. Interval]
Sistan and Baluchestan	Rask	490	66 (13.5%) [10.5-16.8%]	11 (2.2%) [1.1-3.9%]
	Chabahar	500	56 (11.2%) [8.5-14.2%]	0 (0.0%) [0.0-0.73%]
	Sarbaz	469	35 (7.5%) [5.2-10.2%]	0 (0.0%) [0.0-0.78%]
	Konarak	363	16 (4.4%) [2.5-7.0%]	0 (0.0%) [0.0-1.0%]
	Saravan	499	7 (1.4%) [0.5-2.8%]	0 (0.0%) [0.0-0.73%]
	Mirjaveh	200	1 (0.5%) [0.1-2.7%]	0 (0.0%) [0.0-1.8%]
	Zarabad	136	0 (0.0%) [0.0-2.7%]	0 (0.0%) [0.0-2.6%]
Hormozgan	Bandar Khamir	440	7 (1.6%) [0.6-3.2%]	0 (0.0%) [0.0-0.83%]
	Minab	710	7 (1.0%) [0.3-2.0%]	0 (0.0%) [0.0-0.51%]
	Jask	447	6 (1.3%) [0.4-2.8%]	0 (0.0%) [0.0-0.82%]
	Bandar Lengeh	1297	5 (0. 4%) [0.1-0.8%]	0 (0.0%) [0.0-0.28%]
	Sirik	514	4 (0.8%) [0.2-1.9%]	0 (0.0%) [0.0-0.71%]
	Hajiabad	430	3 (0.7%) [0.1-2.0%]	0 (0.0%) [0.0-0.85%]
	Rudan	278	3 (1.1%) [0.2-3.1%]	0 (0.0%) [0.0-1.3%]
	Bandar Abas	631	2 (0.3%) [0.03-1.1%]	0 (0.0%) [0.0-0.58%]
	Parsian	32	0 (0.0%) [0.0-10.8%]	0 (0.0%) [0.0-10.8%]
	Qeshm	177	0 (0.0%) [0.0-2.0%]	0 (0.0%) [0.0-2.0%]
Khuzestan	DashtAzadegan	501	15 (3%) [1.6-4.8%]	0 (0.0%) [0.0-0.73%]
	Ahvaz	500	7 (1.4%) [0.5-2.8%]	0 (0.0%) [0.0-0.73%]

	Bandar-e Mahshahr	471	6 (1.3%) [0.4-2.7%]	0 (0.0%) [0.0-0.78%]
	Hendijan	500	3 (0.6%) [0.1-1.7%]	0 (0.0%) [0.0-0.73%]
	Khoramshahr	40	1 (2.5%) [0.06-13.1%]	0 (0.0%) [0.0-8.8%]
	Abadan	59	0 (0.0%) [0.0-6.0%]	0 (0.0%) [0.0-6.0%]
Bushehr	Bushehr	136	3 (2.2%) [0.45-6.3%]	0 (0.0%) [0.0-2.6%]
	Asaluyeh	14	0 (0.0%) [0.0-23.1%]	0 (0.0%) [0.0-23.1%]
	Kangan	32	0 (0.0%) [0.0-10.8%]	0 (0.0%) [0.0-10.8%]
	Genaveh	140	0 (0.0%) [0.0-2.6%]	0 (0.0%) [0.0-2.6%]
Kerman	Manujan	110	2)1.8%([0.22-6.4%]	0 (0.0%) [0.0-3.2%]
	Jiroft	80	0 (0.0%) [0.0-4.5%]	0 (0.0%) [0.0-4.5%]
	Rudbar-e Jonubi	80	0 (0.0%) [0.0-4.5%]	0 (0.0%) [0.0-4.5%]
	Anbarabad	50	0 (0.0%) [0.0-7.1%]	0 (0.0%) [0.0-7.1%]
	Faryab	14	0 (0.0%) [0.0-23.1%]	0 (0.0%) [0.0-23.1%]
	Qalehganj	27	0 (0.0%) [0.0-12.7%]	0 (0.0%) [0.0-12.7%]
	Kahnooj	80	0 (0.0%) [0.0-4.5%]	0 (0.0%) [0.0-4.5%]
East-Azerbaijan	Jolfa	494	1 (0.2%) [0.005-1.1%]	0 (0.0%) [0.0-0.74%]
West-Azarbaijan	Bazargan	126	0 (0.0%) [0.0-2.8%]	0 (0.0%) [0.0-2.8%]
Guilan	Astara	125	0 (0.0%) [0.0-2.9%]	0 (0.0%) [0.0-2.9%]
Total			256 (2.3%) [2.1-2.6%]	11 (0.1%) [0.05-0.17%]

Table 2. Frequency of DENV IgG and CHIKV virus IgG seropositivity according to demographic variables during 2020-2023 in Iran

CHIKV IgG			DENV IgG				
P value	No. Positive (%)	No. Tested (%)	P value	No. Positive (%)	No. Tested (%)	Type/Name	Variables
0.013	10 (0.2%)	5974	0.644	133 (2.2%)	5974	Female	**Gender**
	1 (0.02%)	5218		123 (2.4%)	5218	Male	
0.562	11 (0.11%)	10860	0.880	248 (2.3%)	10860	Iranian	**Nationality**
	0 (0.0%)	332		8 (2.4%)	332	Non-Iranian	
0.890	0 (0.0%)	232	**<0.001**	1 (0.4%)	232	**<10**	**Age (years)**
	0 (0.0%)	991		13 (1.3%)	991	**10-20**	
	3 (0.1%)	3075		44 (1.4%)	3075	**21-30**	
	4 (0.1%)	2996		83 (2.8%)	2996	**31-40**	
	2 (0.1%)	1566		54 (3.4%)	1566	**41-50**	
	2 (0.1%)	1529		51 (3.3%)	1529	**>50**	
0.277	0 (0.0%)	97	**0.001**	4 (4.1%)	97	Healthcare Worker	**Occupation**
	1 (0.03%)	3498		79 (2.3%)	3498	Self-Employed	
	0 (0.0%)	715		6 (0.8%)	715	Employee	
	10 (0.2%)	4460		122 (2.7%)	4460	Housewife	
	0 (0.0%)	192		11 (5.7%)	192	Farmer	
	0 (0.0%)	159		4 (2.5%)	159	Driver	
	0 (0.0%)	324		2 (0.6%)	324	Student	
	0 (0.0%)	120		3 (2.5%)	120	Military Personnel	
	0 (0.0%)	153		1 (0.7%)	153	Children	
	0 (0.0%)	3		0 (0.0%)	3	Elderly	
	0 (0.0%)	1471		24 (1.6%)	1471	Unknown	

<0.001	0 (0.0%)	188	**<0.001**	0 (0.0%)	188	2020	**Year of Sampling**
	0 (0.0%)	7559		83 (1.1%)	7559	2021	
	11 (0. 4%)	3057		173 (5.5%)	3057	2022	
	0 (0.0%)	388		0 (0.0%)	388	2023	
0.519	11)0.1% (	10784	**<0.001**	249)2.3% (	10784	No	**Abroad Travel History**
	0 (0.0%)	408		67 (16.4% (	408	Yes	

4. DISCUSSION

Our cross-sectional serosurvey of DENV and CHIKV which to our knowledge is the first comprehensive study on the general population of Iran, indicated that 2.3% and 0.1% of the studied population had past exposure to DENV and CHIKV, respectively. The DENV seropositivity was higher in southern provinces than in northern provinces. Only one DENV IgG-positive case was identified in northern Iran (East-Azarbaijan), while all southern provinces showed seropositivity. The highest DENV seroprevalence was observed in Sistan and Baluchestan, Khuzestan, Bushehr and Hormozgan with seropositivity rates of 6.8%, 1.5%, 0.9% and 0.7%. Several previous studies demonstrated the seroprevalence of DENV in southern provinces of Iran. Between 2000 and 2012, a total of 300 serum samples of CCHF-suspected patients from different provinces of Iran were tested for DENV infection and 10 cases (3.3%) were IgG seropositive, of which the majority of cases (n = 4, 40%) were from Sistan and Baluchestan Province and reported no history of travel to abroad [16]. In a 2014 study from Sistan and Baluchestan Province, a seroprevalence rate of 5.9% was reported in Blood donors of Chabahar city. Here, our results showed a higher seropositivity rate of 11.2% in the general population of Chabahar city which indicates an almost two-fold increase in the seroprevalence of DENV from 2020–2023 compared to 2014 [17]. It is worth mentioning that Chabahar port which is close to the Pakistan border has been spotted as one of the main entry points of *Ae. albopictus* into the southern region of Iran since two distinct invasion events by *Ae. albopictus* were reported in this city in 2009 and 2013 [15, 20]. However, because the number of *Ae. albopictus* found in this region was few and the vector did not establish neither in 2009 nor in 2013 in Chabahar, the seropositive cases could not be associated with the identification *Ae. albopictus.*

In a study which was conducted from 2017 to 2018, on 1275 febrile patients from six provinces including Bushehr, Hormozgan, Sistan and Baluchestan, Khuzestan, Guilan and Mazandaran, DENV IgG ELISA results were positive in 5.9% of cases which was higher than the overall DENV seroprevalence in our work (2.3%), possibly due to the difference in the study populations (febrile patients vs. general healthy population) [18].

Regarding CHIKV seroprevalence, interestingly all 11 positive cases were from Rask City (2.2% seroprevalence) in Sistan and Baluchestan Province where the highest DENV seropositivity rate was also documented suggesting that Sistan and Baluchestan is a high-risk area for the spread of both DENV and CHIKV and intensified entomological and human surveillance are highly recommended to be performed in this region for early identification of these arboviruses in vectors and patients. In the border areas, the impact of travel on the communicable disease outbreak should be considered. Iran is seriously threatened by the outbreaks of DENV and CHIKV in Pakistan, especially now that the main vector species are getting ground in the south and north of the country paving the way for local transmission of the diseases should imported cases arrive in number from infected countries. In our study, there was a statistically significant correlation between travelling abroad and DENV seropositivity. However, there was no association between CHIKV seropositivity and travelling abroad. This could be justified by the fact that few CHIKV positive cases were detected in our study. It is noteworthy that some participants from Sistan and Baluchestan Province might not have reported their travel history to Pakistan because they travelled illegally.

Previously, in 2017 a direct association was reported between travel to Pakistan with chikungunya infections among 159 symptomatic patients [21]. It should be considered that during our study volunteers without clinical symptoms were referred, and in the study of Pouriayevali, all 159 specimens had been taken from patients with clinical symptoms suspected of chikungunya. Conversely, the possibility that individuals did not report their travel history correctly could have interfered with identifying the presumed link.

In line with several previous studies that showed an association between age and DENV IgG seropositivity [22–25], in our study, the DENV seropositivity rate was significantly higher in older people (P-value > 00.1).

The concurrent detection of DENV and CHIKV IgG antibodies was observed in 7 cases which can be due to simultaneous infections or possible cross-reactivity in ELISA. Cross-reactivity between DENV and CHIKV has recently been observed using ELISA EUROIMMUN kits [26]. Co-circulation of mosquito-borne viral infections is a public health concern, as clinicians often overlook them, especially in areas with insufficient laboratory capacity. Since the clinical symptoms of CHIKV and DENV infection are similar to several other flavivirus infections, appropriate diagnostic methods to identify the exact virus are necessary. Molecular diagnostic tests can help to rule out serological cross-reactivity and avoid false positive results [26–28].

The main limitation of this study is that the DENV seropositive samples in ELISA should be confirmed by the viral neutralization test due to the possible cross-reactivity with other flaviviruses which could not be done in this study due to the infrastructural issues.

In this study, 188, 7559, 3057 and 388 samples were collected in 2020, 2021, 2022 and 2023, respectively. However, all DENV seropositive cases were observed in 2022 and 2021 and interestingly the positivity rate in 2022 was 5 times higher than 2021 (5.5% vs. 1.1%), indicating an increasing trend over

time. Unfortunately, we could not collect enough samples in 2023 and if a large number of samples were collected in this year, the increasing trend could also be observed in 2023.

4.1 Conclusion

In conclusion, this serosurvey of DENV and CHIKV in the general population of eight provinces in Iran indicated that 2.3% and 0.1% of participants had past exposure to DENV and CHIKV, respectively. The seroprevalence of DENV was significantly higher in southern regions, especially in Sistan and Baluchestan Province. Additionally, CHIKV seropositive cases were solely from the Sistan and Baluchestan Province, emphasizing the requirement of improving integrated surveillance systems followed by envisioning appropriate control measures to keep the vector populations low in all infested provinces with particular attention in higher risk provinces. As more provinces of Iran are invaded by either of the *Aedes* vector species, systematic seroepidemiology studies in high-risk provinces among high-risk human populations with an emphasis on timely identification of acute infections are recommended.

Abbreviations

DENV
Dengue Virus
CHIKV
Chikungunya Virus
OD
Optical Density
SD
Standard Deviation
CCHF
Crimean–Congo Hemorrhagic Fever Disease

Declarations

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Consent for publication: Not applicable.

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Figures

Figure 1

Map of Iran with the location of the studied provinces (in color) where sampling took place

Figure 2. Geographical distribution of DENV IgG and CHIKV IgG seropositive cases by province and city.

West-Azarbaijan province: Bazargan=1. East-Azerbaijan province: Jolfa=2. Guilan province: Astara=3. Khuzestan province: DashtAzadegan=4, Khoramshahr=5, Ahvaz=6, Bandar-e Mahshahr=7, Abadan=8, Hendijan=9. Bushehr province: Genaveh=10, Bushehr=11, Kangan=12, Asaluyeh=13. Hormozgan province: Parsian=14, Bandar Lengeh=15, Bandar Khamir=16, Qeshm=17, Bandar Abas=18, Hajiabad=19, Rudan=20, Minab=21 Sirik=22, Jask=23. Kerman province: Manujan=24, Qalehganj=25, Kahnooj=26, Rudbar-e Jonubi=27, Faryab=28, Jiroft=29, Anbarabad=30. Sistan and Baluchestan province: Zarabad=31, Konarak=32, Chabahar=33, Rask=34, Sarbaz:35, Saravan=36, Mirjaveh=37. ✪= Location of all detected CHIKV IgG seropositive cases

Figure 2

See image above for figure legend