



ELSEVIER

Contents lists available at ScienceDirect

IJID Regions

journal homepage: [www.elsevier.com/locate/ijregi](http://www.elsevier.com/locate/ijregi)

## Human herpes simplex virus-6 (HHV-6) detection and seroprevalence among Qatari nationals and immigrants residing in Qatar

Duaa W. Al-Sadeq<sup>a,b,1</sup>, Hadeel T. Zedan<sup>a,c,1</sup>, Nader Aldewik<sup>d</sup>, Alaa Elkhider<sup>c</sup>, Asalet Hicazi<sup>c</sup>, Nadin Younes<sup>c</sup>, Houssein H. Ayoub<sup>e</sup>, Laith Abu Raddad<sup>f</sup>, Hadi M. Yassine<sup>a,c,\*</sup>, Gheyath K. Nasrallah<sup>a,c,\*\*</sup>

<sup>a</sup> Biomedical Research Centre, QU Health, Qatar University, Doha, Qatar

<sup>b</sup> College of Medicine, QU Health, Qatar University, Doha, Qatar

<sup>c</sup> Department of Biomedical Science, College of Health Sciences, QU Health, Qatar University, Doha, Qatar

<sup>d</sup> Clinical and Metabolic Genetics Section, Paediatrics Department, Hamad General Hospital, Women's Wellness and Research Centre, Interim Translational Research Institute, Hamad Medical Corporation, College of Health and Life Science, Hamad Bin Khalifa University, Doha, Qatar

<sup>e</sup> Mathematics Programme, Department of Mathematics, Statistics and Physics, College of Arts and Sciences, Qatar University, Doha, Qatar

<sup>f</sup> Infectious Disease Epidemiology Group, Weill Cornell Medicine-Qatar, Cornell University, Doha, Qatar

### ARTICLE INFO

**Keywords:**  
HHV-6  
Seroprevalence  
Blood donors  
Viraemia  
Transfusion

### ABSTRACT

**Background:** Human herpes simplex virus-6 (HHV-6) is the causative agent of exanthema subitum. Transmission mainly occurs through salivary secretions, yet blood transfusions and organ transplantations have also been reported as routes of transmission. Studies of seroprevalence of HHV-6 in the Middle East and North Africa (MENA) region and other parts of Asia are scarce. As such, this study aimed to estimate the seroprevalence of HHV-6 among healthy blood donors in Qatar.

**Methods:** In total, 620 healthy blood donors from different nationalities residing in Qatar, mainly from the MENA region and Southeast Asia, were tested using a commercial anti-HHV-6 immunoglobulin G (IgG) enzyme-linked immunosorbent assay kit. In addition, HHV-6 DNA from randomly selected samples was tested and quantified using quantitative reverse transcriptase polymerase chain reaction.

**Results:** Anti-HHV-6 IgG was detected in 71.7% (445/620) [95% confidence interval (CI) 68.2–75.3%] of the tested samples, while 24.3% (61/251) (95% CI 20.0–29.6%) had detectable HHV-6 viraemia. Only 22.5% of individuals with positive IgG status had detectable HHV-6 DNA in their blood, indicating a weak association between viraemia and IgG positivity ( $P=0.08$ ). Furthermore, no significant difference was associated between HHV-6 viraemia and demographic characteristics, except for nationality.

**Conclusion:** The seroprevalence of HHV-6 in Qatar was found to be similar to rates reported in other parts of the world.

### Introduction

Human herpes simplex virus-6 (HHV-6) is a double-stranded linear DNA virus that belongs to the genus *Roseoloviruses* of the *Herpesviridae* family (De Bolle et al., 2005). HHV-6 was first isolated from patients with lymphoproliferative disorders in 1986, and is associated with fatal diseases (Salahuddin et al., 1986). Most people become infected with HHV-6 by the age of 1–2 years (Agut et al., 2015). HHV-6 has two identified variants: HHV-6A, which occurs more frequently in immunocompromised hosts; and HHV-6B, which is the aetiologic agent of exanthema subitum (Caserta et al., 2001). Exanthema subitum, also known as the

‘sixth disease’, is a paediatric disease mainly caused by primary infection with HHV-6 and a minor contribution from HHV-7 (Yamanishi et al., 1988). The disease is usually present in children aged 6 months to 3 years, with an incubation period of 1–2 weeks (Tesini et al., 2014). Exanthema subitum is characterized by high fever, reaching 40°C within 5 days. Later, the patient develops a mild rash on the neck, trunk and face. Primary HHV-6 infection is among the most prevalent causes of acute febrile illness in young children (Caserta et al., 2001). After the primary acute infection, HHV-6 establishes lifelong latency in which the viral genome is maintained and distributed to daughter cells without production of infectious virus, and viral gene expression is limited (low level of

\* Corresponding authors. H.M. Yassine, Biomedical Research Centre, QU Health, Qatar University, P.O. Box 2713, Doha, Qatar.

\*\* G.K. Nasrallah, Department of Biomedical Science, College of Health Sciences, QU Health, Qatar University, P.O. Box 2713, Doha, Qatar.

E-mail addresses: [hyassine@qu.edu.qa](mailto:hyassine@qu.edu.qa) (H.M. Yassine), [gheyath.nasrallah@qu.edu.qa](mailto:gheyath.nasrallah@qu.edu.qa) (G.K. Nasrallah).

<sup>1</sup> These authors contributed equally.

<https://doi.org/10.1016/j.ijregi.2021.12.005>

Received 18 November 2021; Received in revised form 9 December 2021; Accepted 10 December 2021

2772-7076/© 2021 The Authors. Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

chronic viral replication) (Pantry and Medveczky, 2017). HHV-6 latency has been established in the monocyte/macrophage cell population, bone marrow progenitor cells and T cells (Pantry and Medveczky, 2017; Rebecchi et al., 2021). As such, it has been associated with chronic lymphoproliferative syndromes (Straus, 1988). HHV-6 can reactivate intermittently, causing symptoms such as unexplained fever, rash, hepatitis, pneumonitis, encephalitis and bone marrow suppression (Wang et al., 2021b). HHV-6 can be the causative agent of certain diseases, and can be a cofactor in the prognosis of conditions such as heterophile-negative infectious mononucleosis-like illness (non-Epstein-Barr virus and non-cytomegalovirus), viral hepatitis (non-A, B or C), meningitis, encephalitis, acquired immunodeficiency syndrome, lymphoproliferative disorders, and many other clinical conditions.

HHV-6 is transmitted through saliva and secretions from the respiratory tract. Other routes of transmission, such as blood transfusion (Salahuddin et al., 1986) and organ transplantation, have also been reported (Deborska-Materkowska et al., 2006; Weinberg et al., 2005). In antibody-positive or immunocompetent recipients of blood donation, the infected transfused white blood cells carrying latent integrated HHV-6 are eliminated by the recipient's immune system (Politou et al., 2014). However, in immunocompromised patients, especially those who have received stem cell transplantation, there is an existing possibility that the integrated HHV-6 virus in the transplanted haematopoietic cells can be reactivated and lead to acute infection and severe complications (Wilborn et al., 1994b; Agut et al., 2015). Most importantly, HHV-6 reactivation has increasingly been associated with acute graft-vs-host disease (aGVHD) and allograft rejections in organ transplantation settings (Yoshihara et al., 2004; Phan et al., 2018; Wang et al., 2021a). For instance, following bone marrow transplantation, recent evidence has shown an association between HHV-6 and fever and rash resembling aGVHD (Asano et al., 1991; Wilborn et al., 1994a; Appleton et al., 1995; Takemoto et al., 2000), interstitial pneumonitis (Carrigan et al., 1991; Cone et al., 1993), encephalitis (Drobyski et al., 1994), cytomegalovirus disease (Kadokia et al., 1996) and bone marrow suppression (Drobyski et al., 1993; Wang et al., 1996; Ljungman et al., 2000). In addition, nearly 10% of all cord blood transplant recipients develop HHV-6 encephalitis, compared with only 1% of stem cell transplant recipients (Scheurer et al., 2013). Moreover, a recent survey of 235 allogeneic stem cell transplant patients indicated that post-transplant HHV-6 reactivation was strongly associated with the development of aGVHD, delayed platelet engraftment and early post-transplantation mortality (Dulery et al., 2012).

The diagnosis of HHV-6 infection is performed by serology (indirect method) or real-time polymerase chain reaction (PCR), viral culture, in-situ hybridization and immunohistochemistry (direct methods). However, the most prominent technique is the quantification of viral DNA in blood, other body fluids and organs by means of real-time PCR. Although real-time PCR is useful for HHV-6 diagnosis and determining viral load, serological tests such as immunofluorescence and enzyme-linked immunosorbent assays (ELISAs) have the potential to differentiate latent from lytic infection, and can detect past exposure (Burbelo et al., 2012). For patients experiencing primary or acute infection, serologic studies have shown the appearance of specific immunoglobulin M (IgM) antibodies during the first 7 days of infection, reaching maximum titres within 2–3 weeks, and then declining to undetectable levels by 3 months. IgG antibodies appear just after IgM antibodies but persist indefinitely (LaCroix et al., 2000).

Blood-banking organizations adopt strict regulations to minimize the risk of transfusion transmission of pathogens. However, concerns remain regarding the transmission of untested pathogens, including HHV-6. Therefore, blood-banking organizations need to consider the epidemiology of infection among the population. There are few studies of the seroprevalence of HHV-6 in the Middle East and North Africa (MENA) region and other parts of Asia among healthy blood donors. Previously, the present authors reported a higher incidence of various infectious disease agents among residing expatriates compared with local nationals

(Qatari). This included but was not limited to hepatitis B virus (Al Romaihi et al., 2019), hepatitis C virus (Bener et al., 2009), hepatitis E virus (Al Abisi et al., 2021), West Nile virus (Dargham et al., 2021), dengue virus (Humphrey et al., 2019), chikungunya virus (Humphrey et al., 2019) and others. Similarly, high levels of HHV-6 may be circulating in Qatar. Therefore, this study investigated the estimated HHV-6 infection rate related to age, gender and ethnicity among healthy blood donors residing in Qatar. The information gained will enable health organizations in Qatar to develop new policies aiming to reduce the risk of transmissible diseases related to blood transfusion.

## Materials and methods

### Sample collection and ethical compliance

Samples were collected from healthy blood donors for use in previous seroprevalence studies (Nasrallah et al., 2017, 2018, 2020; Dargham et al., 2018). They were collected anonymously along with all necessary demographic information, including age, nationality and gender. This study was approved by Qatar University Institutional Review Board (QU-IRB 518-EA/15) prior to sample collection. Therefore, this research posed no risk to subjects, and their rights and welfare were not harmed.

The study sample consisted of Qatari and expatriate blood donors aged  $\geq 18$  years ( $n=620$ ) from different origin countries, including MENA and non-MENA nationals. The origin countries included Qatar ( $n=204$ ), Egypt ( $n=87$ ), Syria ( $n=86$ ), India ( $n=55$ ), Jordan ( $n=29$ ), Lebanon ( $n=12$ ), Pakistan ( $n=16$ ), Palestine ( $n=26$ ), Philippines ( $n=11$ ), Sudan ( $n=13$ ), Iran ( $n=8$ ) and Yemen ( $n=10$ ). The demographic characteristics of the donors are summarized in Table 1.

### Detection of anti-HHV-6 IgG using ELISA

In total, 5  $\mu$ L of each patient's serum was used to test for the presence of anti-HHV-6 IgG. Testing was performed using a commercial ELISA kit (ELISA-VIDITEST, Catalogue # ODZ-235; VIDIA, Prague, Czech Republic). The strips are coated with native HHV-6 antigen that can form an antigen-antibody complex in the presence of HHV-6 IgG in the tested serum. This can be detected subsequently by adding an animal anti-human IgG antibody combined with horseradish peroxidase that emits colour following reaction with the chromogenic substrate (TMB-O). This kit is CE-marked for in-vitro diagnosis with sensitivity of 99% and specificity of 95%, as determined by the manufacturer (VIDIA, 2021). Absorbance was read at 450/620 nm using a microplate reader (Epoch 2 Microplate spectrophotometer; BioTek, Winooski, VT, USA).

Qualitative evaluation was achieved by calculating the mean absorbance of the standard and calculating the cut-off value. Using Excel (Microsoft Corp, Redmond, WA, USA), the positivity index for each sample was computed using the following formula: sample positivity index = sample absorbance/cut-off value. The absorbance of samples was categorized into three intervals:  $<0.9$  was interpreted as negative,  $0.90$ – $1.10$  was interpreted as borderline, and  $>1.10$  was interpreted as positive. All borderline samples were retested in duplicate in accordance with the manufacturer's instructions, and the average was taken. The result was considered positive if it remained borderline after repetition.

### HHV-6 DNA detection by real-time PCR

After identifying the HHV-6 IgG-positive samples, the authors were interested in determining the immune status and serological profile of these samples. Therefore, 251 samples were selected at random to test for HHV-6 viraemia. To increase the yield of extracted viral DNA, DNA was extracted from buffy coat samples using a Qiagen kit (Catalogue # 51106; Qiagen, Hilden, Germany). According to the manufacturer's instructions, detection and copy number quantification of HHV-6 DNA in

**Table 1**  
Demographic characteristics of randomly selected blood donor samples ( $n=620$ ).

Characteristic		$n$ (%)
Gender	Female	14 (2.26)
	Male	606 (97.7)
Age (years)	15–24	37 (5.97)
	25–34	241 (38.9)
	35–44	207 (33.4)
	45–54	103 (16.6)
	≥55	32 (5.16)
Nationality	Qatari	204 (32.9)
	Non-Qatari	416 (67.1)
	Egyptian	87 (14.0)
	Syrian	86 (13.9)
	Indian	55 (8.87)
	Jordanian	29 (4.68)
	Palestinian	26 (4.19)
	Pakistani	16 (2.58)
	Sudanese	13 (2.10)
	Lebanese	12 (1.94)
	Philippine	11 (1.77)
	Yemeni	10 (1.61)
	Iranian	8 (1.29)
Other <sup>a</sup>	63 (10.2)	

<sup>a</sup> Included Kuwait, United Arab Emirates, Kingdom of Saudi Arabia, Oman, Bahrain, Iraq, Morocco, Algeria, Libya, Tunisia, Turkey, Bosnia, Cyprus, Greece, Russia, Malaysia, Bangladesh, Nepal, Burma, Singapore, USA, Canada, Spain, Bulgaria, Ireland, UK, Kenya, Somalia, France, Italy, Romania, Germany, Colombia, Brazil, New Zealand, Hungary, the Netherlands, Croatia, Ecuador, Serbia, Macedonia, Sweden, Australia, South Africa, Eritrea, Burkina Faso, Djibouti, Chad, Tanzania and Ethiopia.

all samples were performed using a real-time PCR detection and quantification kit (Reference # Z-Path-HHV6; Primerdesign Ltd, Chandler's Ford, UK). The detection principle of this assay is based on the use of real-time amplification with fluorescent reporter dye probes specific for HHV-6 DNA. Fluorescent dyes were detected using a QuantStudio 6 Flex real-time PCR reader (Applied Biosystems, Waltham, MA, USA). The reaction was considered valid if the internal control had an amplification curve while no amplification was detected in the negative control. The sample was considered RT-PCR positive for HHV-6 if HHV-6 DNA was detected in the tested sample.

#### Statistical analysis

Age was categorized into 10-year cohorts: 15–24, 25–34, 35–44, 45–54 and ≥55 years. Overall, age-specific, nationality-specific and gender-specific seroprevalence measures of HHV-6 IgG were estimated. Descriptive statistics [mean, standard deviation (SD)] were used to investigate the characteristics of the study sample. In addition, Pearson's Chi-squared test was used to perform a bivariate analysis for the association between HHV-6 status and potential associated factors. A multi-variate logistic regression model was also performed to study the association between antibody status (dependent variable) and all independent variables, including gender, age and nationality. Finally, logistic regressions were conducted to estimate and report odds ratios and their 95% confidence intervals (CI). The significance level was defined as  $P=0.05$ . Data were analysed using Statistical Package for the Social Sciences Version 25 (IBM Corp., Armonk, NY, USA).

## Results

### Demographic characteristics of the donors

Six hundred and twenty samples from blood donors residing in Qatar were analysed in the current study, of which 606 (97.7%) were from males and 14 (2.30%) were from females (Table 1). Most samples were obtained from non-Qatari residents (67.1%), and the remaining samples were obtained from Qatari nationals (32.9%). The age of donors ranged between 19 and 65 years [mean 37 (SD 10) years]. Thus, approximately 39% of the donors were aged 25–34 years (Figure 1).

### Seroprevalence of HHV-6 among healthy blood donors in Qatar

In total, 620 samples were serologically tested to determine the seroprevalence of HHV-6 IgG antibodies among the healthy blood donors from different nationalities. Of these, 71.7% of samples were IgG seropositive. Associations between HHV-6 seroprevalence and gender, geographic background and age of the studied individuals were calculated using Chi-squared test (Table 2). No significant association ( $P=0.580$ ) was reported between HHV-6 seroprevalence and gender, probably due to the difference in sample size between the two groups.

Considering that Qatar is a diverse country with expatriates constituting more than 85% of the total population, the difference in HHV-6 seroprevalence between Qataris ( $n=204$ ) and non-Qataris ( $n=416$ ) was investigated. No significant difference in HHV-6 seroprevalence was found between Qataris and non-Qataris ( $P=0.506$ ), with 75.5% of Qatari donors found to be seropositive compared with 70.0% of non-Qatari donors. Similarly, correlation between HHV-6 seroconversion and donors' age was investigated. Although no significant differences were reported between the different age groups ( $P=0.519$ ), infection rates were found to decrease with age. HHV-6 seropositivity ranged from 81.0% in donors aged <25 years to 65.6% in donors aged >55 years, suggesting that most people acquire the infection and develop antibodies at early ages. Multi-variate logistic regression was conducted to measure associations between the dependent suspected factors and HHV-6 status; no significant differences were found between groups, except that donors of Syrian nationality may have higher seroprevalence ( $P=0.040$ ) (Table 2).

### HHV-6 viraemia rates among healthy blood donors

HHV-6 viral detection and viral load were determined in 251 blood samples selected at random using quantitative reverse transcriptase PCR. HHV-6 viraemia was detected in 24.3% ( $n=61$ ) of samples. No significant correlation was reported between viraemia rates and age (15–24 years, 33.3%; 25–34 years, 26.5%; 35–44 years, 23.7%; 45–54 years, 40.0%; <55 years, 10%). However, a significant association was found between viraemia rates and nationality ( $P=0.04$ ) among tested donors (Table 3). Viral load in the positive samples ranged between 0.03 and 29,727 copies/mL of blood, with a mean of 608.1 copies/mL (95% CI 540.3–1757). Two samples had a very high viral load (1825 and 29,727 copies/mL) and positive IgG status, suggesting acute infection or reactivation. The correlation between ELISA serological data and HHV-6 viraemia in blood was investigated. Results showed that 22.5% of individuals with positive IgG status had detectable HHV-6 viraemia in their blood. In addition, 4.3% of the samples were PCR positive and seronegative for HHV-6, while 52.2% were HHV-6 IgG positive and had no detected viraemia, suggesting a weak association between DNA and IgG positivity (data not shown).

## Discussion

HHV-6 is known to cause exanthema subitem and establishes lifelong latency. However, a few studies have reported that HHV-6 could be transmitted through infected blood donated by healthy-appearing blood

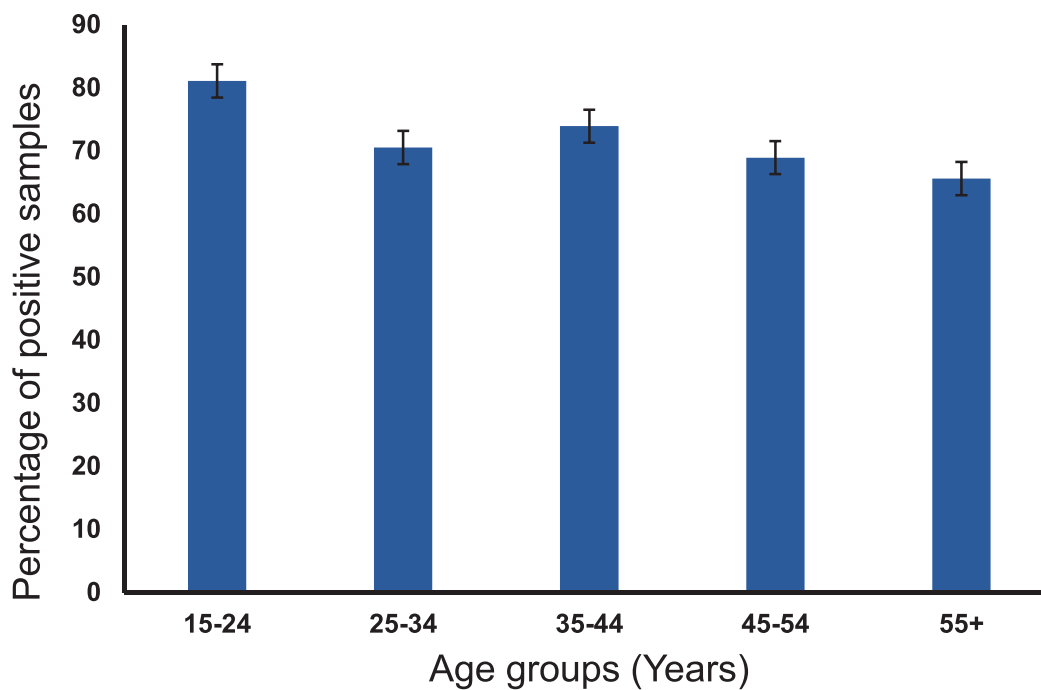


Figure 1. Distribution of human herpes simplex virus-6 (HHV-6) immunoglobulin G-positive blood donations by age group.

Table 2  
Analysis of potential associated factors for human herpes virus-6 among blood donor samples (n=620).

Factor	Bivariate analysis			OR	Multi-variate logistic regression <sup>b</sup>			
	Total n	IgG positive n	P-value <sup>a</sup> (%)		95% CI	Adjusted P-value		Upper limit
Gender								
Male	606	432	71.3	0.076	1			
Female	14	13	92.9		4.70	0.58	38.0	0.147
Nationality								
Qatari	204	154	75.5	0.506	1			0.525
Non-Qatari	416	291	70.0					
Egyptian	87	67	77.0		1.03	0.56	1.88	0.929
Syrian	86	55	64.0		0.56	0.32	0.98	0.043
Indian	55	35	63.6		0.58	0.30	1.10	0.095
Jordanian	29	18	62.1		0.56	0.25	1.27	0.165
Palestinian	26	20	76.9		1.15	0.43	3.03	0.785
Pakistani	16	11	68.8		0.67	0.22	2.04	0.476
Sudanese	13	11	84.6		1.89	0.40	8.86	0.420
Lebanese	12	8	66.7		0.60	0.17	2.13	0.430
Philippine	11	7	63.6		0.46	0.12	1.71	0.244
Yemeni	10	6	60.0		0.48	0.13	1.77	0.267
Iranian	8	6	75.0		0.97	0.19	5.08	0.975
Other nationalities	63	47	74.6		0.92	0.48	1.76	0.792
Age group (years)								
15–24	37	30	81.1	0.519	1			0.676
25–34	241	170	70.5		0.73	0.30	1.80	0.493
35–44	207	153	73.9		0.81	0.33	2.01	0.649
45–54	103	71	68.9		0.63	0.24	1.64	0.345
≥55	32	21	65.6		0.50	0.16	1.55	0.229

IgG, immunoglobulin G; OR, odds ratio; CI, confidence interval.

<sup>a</sup> Pearson Chi-squared test.

<sup>b</sup> Variables entered in the model: gender, nationality and age.

donors (Politou et al., 2014). Although HHV-6 may cause serious complications, donor screening for this virus is not yet mandatory. In addition, only a few studies have investigated the seroprevalence of HHV-6 in healthy populations in the MENA region.

This study revealed overall seroprevalence of HHV-6 IgG of 71.7%, comparable with previous estimates reported elsewhere. For instance, Linhares et al. (1991) reported seroprevalence of 76.5% and 77.2% for Brazilians and Japanese immigrants, respectively. Similarly, anti-

HHV-6 lytic IgG antibodies were analysed in Greece using commercially available ELISA kits. The study reported seroprevalence of HHV-6 of 78.75%, without any significant difference between males and females (Politou et al., 2014). In addition, a study in Egypt reported seroprevalence of HHV-6 IgG of 75% (Assem et al., 2005). Moreover, based on immunofluorescence assay detection of HHV-6 IgG in Kuala Lumpur, seroprevalence was 83% with no significant association between different ethnic groups, such as Chinese, Malays and Malaysia Indians

**Table 3**  
Human herpes virus-6 viraemia in the studied population (n=251).

Category	Positive/total n	Prevalence (95% CI)	P-value <sup>a</sup>
Gender			
Male	53/224	23.7 (18.1–29.2)	0.997
Female	0/4	0	
Nationality			
Qatari	3/39	7.7 (0.6–16.1)	
Non-Qatari	50/186	26.9 (20.5–33.3)	0.037
Age group (years)			
15–24	3/9	33.3 (25.3–41.1)	
25–34	22/83	26.5 (17.0–36.0)	0.845
35–44	14/59	23.7 (12.9–34.6)	
45–54	10/25	40 (20.8–59.2)	
> 51	1/10	10 (2.8–18.5)	

CI, confidence interval.

<sup>a</sup> Pearson's Chi-squared test.

(Chua et al., 1996). According to the manufacturer, the specificity of the ELISA-VIDITEST used in the present study is 95%. Thus, the present test results could be slightly overestimated as there is a possibility of cross-reactivity with other herpes and non-herpes viruses, which may have given false-positive results.

Despite an apparent decreasing trend in the seroprevalence of HHV-6-IgG antibodies with age in the current study, the trend was not found to be statistically significant. On the other hand, a significant association was observed between HHV-6 viraemia and nationality, indicating evidence of variation in HHV-6 infection exposure by nationality. Seroprevalence was slightly lower among blood donors residing in Qatar who were MENA nationals compared with Qataris. Nevertheless, the seroprevalence of HHV-6 was highest in Sudanese and Egyptians. This was expected as the main route of transmission is through saliva. Additionally, sharing utensils, which could include saliva, would play a considerable role in transmitting the virus (Wojcicki, 2003).

The reported high seroprevalence of HHV-6 IgG could be due to primary infection and the persistence of IgG for long-life latency or reactivation of the virus in immunocompromised patients. Thus, many factors should be considered, such as history of transplantation or cases of severe drug hypersensitivity symptoms. Many studies have shown that HHV-6 can reactivate among patients who have drug-induced hypersensitivity syndrome that leads to high HHV-6 IgG or DNA viraemia (Aihara et al., 2003).

When HHV-6-IgG is detected after convalescence, this indicates virus reactivation (Denes et al., 2004). However, reactivation needs to be confirmed by detection of HHV-6 viraemia. Therefore, the combination of quantitative PCR and serology is increasingly recommended for HHV-6 diagnosis to differentiate recent infections from past infections. In this study, 24.3% of the tested samples had a detectable HHV-6 viral load measured by real-time PCR. Similar rates of HHV-6 infection have been reported in patients with chronic hepatitis C virus infection (Ibrahim et al., 2018). Moreover, a study was conducted to evaluate the prevalence of HHV-6 IgG using ELISA and PCR, of which 107/112 (96.4%) were positive for HHV-6 IgG, while 6/112 (5.4%) were positive for HHV-6 viraemia. In another study, 401 randomly selected consecutive blood donors were tested for HHVs viraemia. Results showed that 3.49% of blood specimens had detectable HHV-6 viraemia, with viral load reaching 1,580,400 copies per mL (Rouka and Kyriakou, 2015). In the present study, positive HHV-6 viraemia samples were considered if viral DNA was detected in the sample. The average viral load was 608 copies per mL, and the maximum was 29,727 copies per mL. Interestingly, two samples were positive for HHV-6 viraemia with high viral load and positive for HHV-6 IgG, which could indicate HHV-6 reactivation. However, no significant differences in viraemia rates were reported, except between donors from various nationalities.

To the authors' knowledge, this is the first study to define the status of HHV-6 seroprevalence and viraemia among healthy individuals

in Qatar and the Middle East. However, there were some limitations that may have affected the study. Firstly, insufficient patient information was collected from the blood bank. For instance, there was no information on potential contributing factors such as educational level, socio-economic status, travel history, transfusion history, transplantation history and dialysis history, as only minimal information about the donors was provided. Ultimately, this has left gaps in Qatar's public health records. Moreover, the small number of female donors (n=14) compared with male donors (n=606) invalidated the comparison between the two groups. Moreover, the study was limited to samples collected from donors aged >18 years, where most participants were seropositive. Thus, a similar study with a younger group is needed.

In conclusion, to the authors' knowledge, this is the first HHV-6 seroprevalence study to be performed on healthy blood donors in Qatar. In this study, ELISA-VIDITEST kits were used to detect HHV-6 IgG in plasma samples collected from blood donors in Qatar. The existence of donor samples with high HHV-6 IgG raises questions regarding the potential risk of HHV-6 blood-borne infection and the safety of blood products. Furthermore, as HHV-6 is associated with many clinical conditions, this study highlights the importance of blood donation pre-screening, particularly when transfusion to immunocompromised patients is warranted.

### Conflict of interest statement

None declared.

### Funding

This work was made possible by collaborative grant number M-QJRC-2020-5 from Qatar University.

### Ethical approval

This study was conducted in accordance with the guidelines of the Declaration of Helsinki, and was approved by the Institutional Review Board of Qatar University IRB (QU-IRB 518-EA/15)

### Author contributions

Duaa W. Al-Sadeq: development of methodology, use of software, formal analysis, writing, visualization. Hadeel T. Zedan: development of methodology, use of software, formal analysis, performance of experiments, writing, visualization. Nader Aldewik: writing – review and editing. Alaa Elkhider: performance of experiments. Asalet Hicaz: performance of experiments. Houssein H. Ayoub: use of software, formal analysis, validation. Laith Abu Raddad: writing – review and editing. Nadin Younes: writing – review and editing. Hadi M. Yassine: development of methodology, writing – review and editing. Gheyath K. Nasrallah: conceptualization, validation, formal analysis, provision of resources, storage of data, visualization, supervision, project administration, acquisition of funding.

### References

- Agut H, Bonnafous P, Gautheret-Dejean A. Laboratory and clinical aspects of human herpesvirus 6 infections. *Clin Microbiol Rev* 2015;28:313–35.
- Aihara Y, Ito SI, Kobayashi Y, Yamakawa Y, Aihara M, Yokota S. Carbamazepine-induced hypersensitivity syndrome associated with transient hypogammaglobulinaemia and reactivation of human herpesvirus 6 infection demonstrated by real-time quantitative polymerase chain reaction. *Br J Dermatol* 2003;149:165–9.
- Al Absi ES, Al-Sadeq DW, Khalili M, Younes N, Al-Dewik N, Abdelghany SK, et al. The prevalence of HEV among non-A-C hepatitis in Qatar and efficiency of serological markers for the diagnosis of hepatitis E. *BMC Gastroenterol* 2021;21:266.
- Al Romaihi HE, Ganesan N, Farag EA, Smatti MK, Nasrallah GK, Himatt SM, et al. Demographics and epidemiology of hepatitis B in the state of Qatar: a five-year surveillance-based incidence study. *Pathogens* 2019;8:68.
- Appleton AL, Sviland L, Peiris JS, Taylor CE, Wilkes J, Green MA, et al. Human herpes virus-6 infection in marrow graft recipients: role in pathogenesis of graft-versus-host disease. Newcastle upon Tyne Bone Marrow Transport Group. *Bone Marrow Transplant* 1995;16:777–82.

- Asano Y, Yoshikawa T, Suga S, Nakashima T, Yazaki T, Fukuda M, et al. Reactivation of herpesvirus type 6 in children receiving bone marrow transplants for leukemia. *N Engl J Med* 1991;324:634–5.
- Assem M, Gad W, El-Sharkawy N, El-Rouby M, Ghaleb F, Tarek H, et al. Prevalence of anti human herpes virus-6 IgG and its receptor in acute leukemia (membrane cofactor protein: MCP, CD46). *J Egypt Natl Cancer Inst* 2005;17:29–34.
- Bener A, Al-Kaabi S, Derbala M, Al-Marri A, Rikabi A. The epidemiology of viral hepatitis in Qatar. *Saudi J Kidney Dis Transplant* 2009;20:300–6.
- Burbelo PD, Bayat A, Wagner J, Nutman TB, Baraniuk JN, Iadarola MJ. No serological evidence for a role of HHV-6 infection in chronic fatigue syndrome. *American journal of translational research* 2012;4(4):443.
- Carrigan DR, Drobyski WR, Russler SK, Tapper MA, Knox KK, Ash RC. Interstitial pneumonitis associated with human herpesvirus-6 infection after marrow transplantation. *Lancet* 1991;338:147–9.
- Caserta MT, Mock DJ, Dewhurst S. Human herpesvirus 6. *Clin Infect Dis* 2001;33:829–33.
- Chua KB, Khairullah NS, Hooi PS. Seroepidemiology of human herpesvirus 6 in a population seen in the University Hospital, Kuala Lumpur, Malaysia. *Southeast Asian J Trop Med Publ Health* 1996;27:91–5.
- Cone RW, Hackman RC, Huang ML, Bowden RA, Meyers JD, Metcalf M, et al. Human herpesvirus 6 in lung tissue from patients with pneumonitis after bone marrow transplantation. *N Engl J Med* 1993;329:156–61.
- Dargham SR, Nasrallah GK, Al-Absi ES, Mohammed LI, Al-Disi RS, Nofal MY, et al. Herpes simplex virus type 2 seroprevalence among different national populations of Middle East and North African Men. *Sex Transm Dis* 2018;45:482–7.
- Dargham SR, Al-Sadeq DW, Yassine HM, Ahmed M, Kunhipurayil H, Humphrey JM, et al. Seroprevalence of West Nile virus among healthy blood donors from different national populations residing in Qatar. *Int J Infect Dis* 2021;103:502–6.
- De Bolle L, Naesens L, De Clercq E. Update on human herpesvirus 6 biology, clinical features, and therapy. *Clin Microbiol Rev* 2005;18:217–45.
- Deborska-Materkowska D, Lewandowski Z, Sadowska A, Nowacka-Cieciura E, Chudziński W, Czerwiński J, et al. Fever, human herpesvirus-6 (HHV-6) seroconversion, and acute rejection episodes as a function of the initial seroprevalence for HHV-6 in renal transplant recipients. *Transplant Proc* 2006;38:139–43.
- Denes E, Magy L, Pradeau K, Alain S, Weinbreck P, Ranger-Rogez S. Successful treatment of human herpesvirus 6 encephalomyelitis in immunocompetent patient. *Emerg Infect Dis* 2004;10:729.
- Drobyski WR, Dunne WM, Burd EM, Knox KK, Ash RC, Horowitz MM, et al. Human herpesvirus-6 (HHV-6) infection in allogeneic bone marrow transplant recipients: evidence of a marrow-suppressive role for HHV-6 in vivo. *J Infect Dis* 1993;167:735–9.
- Drobyski WR, Knox KK, Majewski D, Carrigan DR. Brief report: fatal encephalitis due to variant B human herpesvirus-6 infection in a bone marrow-transplant recipient. *N Engl J Med* 1994;330:1356–60.
- Dulery R, Salleron J, Dewilde A, Rossignol J, Boyle EM, Gay J, et al. Early human herpesvirus type 6 reactivation after allogeneic stem cell transplantation: a large-scale clinical study. *Biol Blood Marrow Transplant* 2012;18:1080–9.
- Humphrey JM, Al-Absi ES, Hamdan MM, Okasha SS, Al-Trmanini DM, El-Dous HG, et al. Dengue and chikungunya seroprevalence among Qatari nationals and immigrants residing in Qatar. *PLoS One* 2019;14.
- Ibrahim M, Soad W, Alfarouk O, Elsbali A, Al-Dahr S, Mohamed W. Prevalence of human herpes virus-6 (HHV-6) among chronic HCV patients. *Saudi J Pathol Microbiol* 2018.
- Kadokia MP, Rybka WB, Stewart JA, Patton JL, Stamey FR, Elsayy M, et al. Human herpesvirus 6: infection and disease following autologous and allogeneic bone marrow transplantation. *Blood* 1996;87:5341–54.
- LaCroix S, Stewart JA, Thouless ME, Black JB. An immunoblot assay for detection of immunoglobulin M antibody to human herpesvirus 6. *Clinical and diagnostic laboratory immunology* 2000;7(5):823–37.
- Linhares MIS, Eizuru Y, Tateno S, Minamishima Y. Seroprevalence of human herpesvirus 6 infection in Brazilian and Japanese populations in the North-East of Brazil. *Microbiol Immunol* 1991;35:1023–7.
- Ljungman P, Wang FZ, Clark DA, Emery VC, Remberger M, Ringdén O, et al. High levels of human herpesvirus 6 DNA in peripheral blood leucocytes are correlated to platelet engraftment and disease in allogeneic stem cell transplant patients. *Br J Haematol* 2000;111:774–81.
- Nasrallah GK, Al Absi ES, Ghandour R, Ali NH, Taleb S, Hedaya L, et al. Seroprevalence of hepatitis E virus among blood donors in Qatar (2013–2016). *Transfusion* 2017;57:1801–7.
- Nasrallah GK, Dargham SR, Mohammed LI, Abu-Raddad LJ. Estimating seroprevalence of herpes simplex virus type 1 among different Middle East and North African male populations residing in Qatar. *J Med Virol* 2018;90:184–90.
- Nasrallah G, Dargham S, Harfouche M, Abu-Raddad L. Seroprevalence of herpes simplex virus types 1 and 2 in Indian and Filipino migrant populations in Qatar: a cross-sectional survey. *East Mediterr Health J* 2020;26:609–15.
- Pantry SN, Medveczky PG. Latency, integration, and reactivation of human herpesvirus-6. *Viruses* 2017;9:194.
- Phan TL, Pritchett JC, Leifer C, Zerr DM, Koelle DM, Di Luca D, et al. HHV-6B infection, T-cell reconstitution, and graft-vs-host disease after hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2018;53:1508–17.
- Politou M, Koutras D, Kaparos G, Valsami S, Pittaras T, Logothetis E, et al. Seroprevalence of HHV-6 and HHV-8 among blood donors in Greece. *Virol J* 2014;11:153.
- Rebechi MT, Bork JT, Riedel DJ. HHV-6 encephalitis after chimeric antigen receptor T-cell therapy (CAR-T): 2 case reports and a brief review of the literature. *Open Forum Infect Dis* 2021;8:ofab470.
- Rouka E, Kyriakou D. Molecular epidemiology of human Herpesviruses types 1–6 and 8 among Greek blood donors. *Transfus Med* 2015;25:276–9.
- Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, et al. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* 1986;234:596–601.
- Scheurer ME, Pritchett JC, Amirian ES, Zemke NR, Lusso P, Ljungman P. HHV-6 encephalitis in umbilical cord blood transplantation: a systematic review and meta-analysis. *Bone Marrow Transplant* 2013;48:574–80.
- Straus SE. The chronic mononucleosis syndrome. *J Infect Dis* 1988;157:405–12.
- Takemoto Y, Takatsuka H, Wada H, Mori A, Saheki K, Okada M, et al. Evaluation of CMV/human herpes virus-6 positivity in bronchoalveolar lavage fluids as early detection of acute GVHD following BMT: evidence of a significant relationship. *Bone Marrow Transplant* 2000;26:77–81.
- Tesini BL, Epstein LG, Caserta MT. Clinical impact of primary infection with roseoloviruses. *Curr Opin Virol* 2014;9:91–6.
- VIDIA. Anti-HH6 ELISA-VIDITEST. Prague: VIDIA; 2021.
- Wang FZ, Dahl H, Linde A, Brytting M, Ehrnst A, Ljungman P. Lymphotropic herpesviruses in allogeneic bone marrow transplantation. *Blood* 1996;88:3615–20.
- Wang X, Patel SA, Haddadin M, Cerny J. Post-allogeneic hematopoietic stem cell transplantation viral reactivations and viremias: a focused review on human herpesvirus-6, BK virus and adenovirus. *Ther Adv Infect Dis* 2021a;8 20499361211018027.
- Wang X, Patel SA, Haddadin M, Cerny J. Post-allogeneic hematopoietic stem cell transplantation viral reactivations and viremias: a focused review on human herpesvirus-6, BK virus and adenovirus. *Ther Adv Infect Dis* 2021b;8 20499361211018027.
- Weinberg A, Enomoto L, Li S, Shen D, Coll J, Shpall EJ. Risk of transmission of herpesviruses through cord blood transplantation. *Biol Blood Marrow Transplant* 2005;11:35–8.
- Wilborn F, Brinkmann V, Schmidt CA, Neipel F, Gelderblom H, Siegert W. Herpesvirus type 6 in patients undergoing bone marrow transplantation: serologic features and detection by polymerase chain reaction. *Blood* 1994a;83:3052–8.
- Wilborn F, Schmidt CA, Zimmermann R, Brinkmann V, Neipel F, Siegert W. Detection of herpesvirus type 6 by polymerase chain reaction in blood donors: random tests and prospective longitudinal studies. *Br J Haematol* 1994b;88:187–92.
- Wojcicki JM. Traditional behavioural practices, the exchange of saliva and HHV-8 transmission in sub-Saharan African populations. *Br J Cancer* 2003;89:2016–17.
- Yamanishi K, Okuno T, Shiraki K, Takahashi M, Kondo T, Asano Y, et al. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* 1988;1:1065–7.
- Yoshihara S, Kato R, Inoue T, Miyagawa H, Sashihara J, Kawakami M, et al. Successful treatment of life-threatening human herpesvirus-6 encephalitis with donor lymphocyte infusion in a patient who had undergone human leukocyte antigen-haploidentical nonmyeloablative stem cell transplantation. *Transplantation* 2004;77:835–8.