

# Prevalence of Human Parvovirus B19 and Associated Risk Factors in Women with Bad Obstetric History in Babylon Province, Iraq

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## Abstract

**Background:** Parvovirus infection is an important cause of fetal loss during all three trimesters of pregnancy. **Objectives:** This study was conducted to investigate the prevalence, susceptibility rate, and risk factors of B19-V in women aged 15 to 45 in the Al-Hamza region of Babil, Iraq, considering the negative effects of the infection. **Materials and Methods:** Between January 2022 and March 2023, blood samples were taken from 116 women, including those with and those without a history of bad obstetric history (BOH). Enzyme-linked immunosorbent assay (ELISA) was used to test sera taken from the study participants for B19 IgM and immunoglobulin G (IgG) antibodies. Those samples tested positive for IgG B19 were further subjected to polymerase chain reaction amplification and sequencing, targeting partial gene sequences of the nonstructural viral protein 1 (VP1) gene. **Results:** For human parvovirus IgG and IgM, the seropositivity was 44% and 16.5%, respectively. The following variables were associated in this study with greater seroprevalence rates of IgG and IgM, respectively: age 36–45 years (72.7%, 36.4%); abortion (80%, 32.5%); education (82.2%, 33.3%); unemployment (61%, 22.2%); and overcrowding (73.3%, 31.1%). In this case–control study, positive amplicons (700 bp) were detected from 27 samples out of 40 women of childbearing age who tested positive for IgG. Sequence analysis of the partial sequence of the VP1 gene in all positive amplicons was 100% identical with each other and with human parvovirus B19 (NC: 00088.3). According to the current study, about 66% of women are serologically negative and at risk of contracting parvovirus B19. **Conclusion:** Our findings conclude that pregnant women in Iraq, particularly those with BOH, must undergo frequent serological screening for B19-V during all three trimesters and be given the necessary advice to avoid B19-V infection to improve their pregnancy outcomes.

**Keywords:** B19, B19-V, BOH, Iraq, parvovirus, PCR, pregnant

## INTRODUCTION

Human parvovirus (B19-V) infection is a highly contagious childhood disease with a distinctive face rash for children. It is a cause of erythema infectiosum, aplastic crisis, and acute symmetric polyarthropathy in adults and children. It can also cause fetal complications, including spontaneous abortions and hydrops fetalis.<sup>[1]</sup> According to the UK Health Security Agency,<sup>[2]</sup> B19-V is among several viruses, such as cytomegalovirus (CMV), adversely affecting pregnancy outcomes and causing viral congenital infections. B19-V is transmitted principally through respiratory secretions, hand-to-mouth contact, transfusion of blood products, and from mother to fetus.<sup>[3]</sup>

The diagnosis of parvovirus B19 infection is through serological assays. IgM antibodies can usually be measurable within a range of 7–10 days post-viral acquisition and can be detected for 2–3 months after contracting the virus. About 2 weeks post-viral exposure, immunoglobulin G (IgG) titer will usually rise, and the patient will develop lifelong

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immunity.<sup>[1]</sup> The presence of IgM and IgG antibodies is usually diagnostic of recent infections, while samples with IgG+ and IgM- suggest immunity. Samples with both IgG- and IgM- indicate susceptibility, while samples with IgG- and IgM+ suggest recent exposure to the virus.<sup>[1,3]</sup> Parvovirus is a single-stranded deoxyribonucleic acid (DNA) virus that selectively infects and lyses human erythroblasts.<sup>[3]</sup> Parvovirus is autonomous and does not require a helper virus and was, therefore, until recently, it was classified in the genus *Parvovirus*. Human Parvovirus B19 is now classified as a member of the *Erythroparvovirus* genus of the subfamily Parvovirinae.<sup>[4]</sup> Parvovirus B19 has three genotypes (1–3) and at least four subtypes (1a, 1b, 3a, and 3b), according to genome analyses. Currently, three genotypes with four subtypes correlated to genotypes 1 and 3 (a, 1b, 3a, and 3b) have been found, depending on the isolation year and the geographical location. The most prevalent B19-V is Genotype 1, which shows a widespread distribution. Genotype 2 is more common in patients from Europe, whereas Genotype 3 is common in South America, sub-Saharan Africa, and France.<sup>[5,6]</sup> Worldwide, about 2% of all pregnancies are affected by Parvovirus infection. The rate of infection increases significantly during epidemics to between 3% and 20%, with a seroconversion rate of 3%–34%.<sup>[7]</sup>

In Europe, around 60% of pregnant women are resistant to B19-V infection,<sup>[6]</sup> with a prevalence rate of about 1%–5% for pregnant women and with a risk of transmission of 25%–30% to the fetus.<sup>[8,9]</sup> In the United Kingdom, there was a remarkable increase in the incidence of B19-V in laboratory-confirmed cases among women of childbearing age in 2013, 2017, and 2018.<sup>[10]</sup> In the surrounding regions, B19 was detected among healthy blood donors of different nationalities residing in Qatar,<sup>[11]</sup> Iran,<sup>[12]</sup> and pregnant women in Marrakesh.<sup>[13]</sup> In Iraq, human parvovirus B19 has been recently detected in patients suffering from thalassemia<sup>[14]</sup> and hemophilia A.<sup>[15]</sup> Recent studies have detected the seroprevalence of human parvovirus among pregnant women in Kirkuk,<sup>[16]</sup> Erbil,<sup>[17]</sup> and the southern region of ThiQar.<sup>[18]</sup> There is currently no licensed vaccine and no preventive measures to date. Several studies have previously investigated B19-V among different target groups; however, there are paucity of data on the molecular and seroprevalence of human parvovirus B19 and associated risk factors in women with bad obstetric history (BOH) history, specifically in the current study area of Babil. Bearing in mind the significant consequences of the infection, this study was undertaken to determine the rate of Parvovirus B19 infection in women of childbearing age with BOH and the impact of sociodemographic variables among the target group in the Al-Hamza district of Babil, Iraq.

## MATERIALS AND METHODS

### Study design

The total subjects of this descriptive case–control study involved 116 women of childbearing age (15 to 45 years

old). Women with BOH were recognized and recruited from the primary healthcare centers located in the Al-Hamza district in Babil, Iraq. Twenty-five participants were excluded due to missing data. Of the residual ( $n = 91$ ) study population members, 40 (44%) had BOH and were categorized as research group subjects, 51 (56%) had an average to good obstetric history and were classified as control group members.

### Collection of data

For sample collection, members of the project team regularly visited primary health care centers in the Al-Hamza district. Possible participants were recognized, approached, interviewed, and involved in the project after obtaining informed consent. The exclusion criteria applied for the candidates were as follows: women with uterine or cervical abnormalities, chromosomal abnormalities in either spouse, hyperthyroidism, diabetes mellitus, hypertension, hypothyroidism, renal disorders, or a history of fetal chromosomal or genetic anomalies. The study population was classified into four classes; the first represents pregnant women with a good obstetric history. The second represents pregnant women with BOD. The third represents non-pregnant women with an average to good obstetric history. The fourth class represents non-pregnant women with BOD.

### Collecting and handling of blood samples

A volume of approximately 10 mL of venous blood was taken from each individual between January 2022 and March 2023, and it was stored in sterile containers under aseptic conditions. After this, serum was divided and stored in aliquots at  $-20^{\circ}\text{C}$  until it was processed further.

### Enzyme-linked immunosorbent assay (ELISA) assay

Serum specimens from all participants were analyzed for B19 IgM and IgG antibodies using Vircell ELISA kits. The kit was purchased from Vircell S.L. Prague, Spain, G1031 and M1031 for IgG and IgM, respectively. The ELISA tests were performed in compliance with the manufacturer's recommendations from CALABIOTECH. A microwell ELISA reader was utilized to process the data, and an optical density of 450 nm was utilized for reading.

### DNA extraction

Utilizing the Favor Prep Genomic DNA Mini Kit (Intronbio, Korea), viral genomic DNA was obtained from serum samples in a final total volume of 100  $\mu\text{L}$ , as per the manufacturer's instructions. We measured the amount and quality of DNA using a NanoDrop 2000 spectrophotometer. Until it was needed, the eluted genomic DNA was kept at  $-20^{\circ}\text{C}$ .

**Table 1: Seroprevalence of B19-V immunoglobulin G among study population in regard to age, abortion, education, employability, and residential status**

	Age groups				Abortion status		Education status		Occupation status		Residential status	
	Total	15–25 years old	26–35 years old	36–45 years old	Not aborted	Aborted	Uneducated	Educated	Unemployed	Employed	Not crowded	Crowded
<b>B19-V IgG –ve</b>	51	23	25	3	43	8	43	8	21	30	39	12
	56.0%	62.2%	58.1%	27.3%	84.3%	20.0%	93.5%	17.8%	38.9%	81.1%	84.8%	26.7%
<b>B19-V IgG +ve</b>	40	14	18	8	8	32	3	37	33	7	7	33
	44.0%	37.8%	41.9%	72.7%	15.7%	80.0%	6.5%	82.2%	61.1%	18.9%	15.2%	73.3%
<b>Pearson Chi-square</b>			4.33		<b>37.64</b>		<b>52.91</b>		<b>15.86</b>		<b>31.18</b>	
<b>P value</b>			<i>NS</i>		<b><i>&lt;0.01</i></b>		<b><i>&lt;0.01</i></b>		<b><i>&lt;0.01</i></b>		<b><i>&lt;0.01</i></b>	

IgG, immunoglobulin G

NS refers to *P* values with insignificant difference

Bold values indicated a significant difference

Bold italic values indicate *P* values with a significant difference

### Polymerase chain reaction

The partial gene sequence of the viral protein 1 (VP1) gene was amplified using the MacroGen, Korea, primers (K-1, 5'-ATAAATCCATATACTCATT-3' and K-2, 5'-CTAAAGTATCCTGACCTTG-3'), in compliance with the protocol.<sup>[19]</sup> Using the Maxime PCR PreMix Kit (i-Taq) (Intronbio, Korea), DNA fragments were amplified using the polymerase chain reaction (PCR) in a total volume of 20  $\mu$ L. The PCR tube containing Taq DNA polymerase, dNTP mixture, and reaction buffer was filled with 4  $\mu$ L of template DNA, 2  $\mu$ L of each forward and reverse primer, and 7  $\mu$ L of RNase-free water. The target DNA was amplified using conventional PCR under the parameters given by Loy *et al.* (2002): annealing at 37°C for 120s and elongation at 72°C for 180s for 35 cycles. Positive control was provided by the Central Health Laboratory of the Ministry of Health, Iraq.

### Agarose gel electrophoresis

PCR products (5  $\mu$ L) were separated on a 0.7% (w/v) agarose gel by electrophoresis using 1  $\times$  tris-borate-EDTA buffer containing 0.5  $\mu$ g/mL of simply safe dye, run for 1 h and 30 min, and visualized under 320nm ultra violet light. The size of the products was ascertained by means of comparison with Intronbio (Korea)'s 100bp DNA ladder.

### Sequencing

As described previously elsewhere,<sup>[20]</sup> positive PCR amplicons were gel purified and sequenced by a commercial service. Sequencing was conducted using the same forward and reverse primers used in the PCR. Upon downloading the sequences from the Eurofins Homepage, sequences were further compared with each other by the STADEN Package version 2003.0 Pregap4 and Gap4 programs as described earlier by Bonfield *et al.* (1995).<sup>[21]</sup> BLASTN and BLASTX options from the National Center for Biotechnology Information (NCBI) were used to compare

the obtained sequences with data in GenBank. According to Hall (1999),<sup>[22]</sup> the BioEdit Sequence Alignment Editor program's ClustalW algorithm carried out several alignments of nucleotide sequences.

### Data analyses

The data was collected and assembled using a Microsoft Excel spreadsheet. The relation between variables was examined using a Chi-squared test via SPSS (V.24). In order to calculate the odds ratio, the relation between categorical data was conducted by bivariate regression-line analysis.

### Ethical approval

The Biotechnology ethics committee of Al-Qasim Green University, College of Medical Biotechnology, approved the research proposal to be conducted in its presented form.

## RESULTS

For this study, 116 women in total were pooled. For 91 of these women in the study sample, full data were documented. The research subjects were classified and subcategorized by sociodemographic variables; for example, education, employment, age, or living in overcrowded households. Overall, this study reported an infection rate of 44% with B19-IgG among the study population. Of the three different age groups illustrated in Table 1, IgG seropositivity increased with age, rising from 37.8% in the 15–25 years old to 41.9% in the 26 to 35 years old to reach the highest 72.7% among the 36–45-year old. There is, however, no significant correlation between B19-IgG and age group.

The aborted group (study group) had a higher seroprevalence of B19-IgG seropositivity of 80.0% compared to 15.7% in the non-aborted group (control group). The relation between B19-IgG and abortion status was significant ( $P < 0.01$ ). The prevalence of B19-IgG among the unemployed was highest (61.1%) compared

to the employed (18.9%). The Chi-squared values and *P*-value between B19-IgG and employment status indicate a significant correlation at a *P*-value of <0.01 at a 95% confidence interval (CI). Education and overcrowding cohorts had a B19-IgG seroprevalence of 82.2% and 73.3% compared to the uneducated group (6.5%) and those living alone or in less crowded situations (15.2%). The relation between B19-IgG and education was significant (*P* < 0.01), and there was also a significant correlation between B19 and the crowding situation (*P* < 0.01).

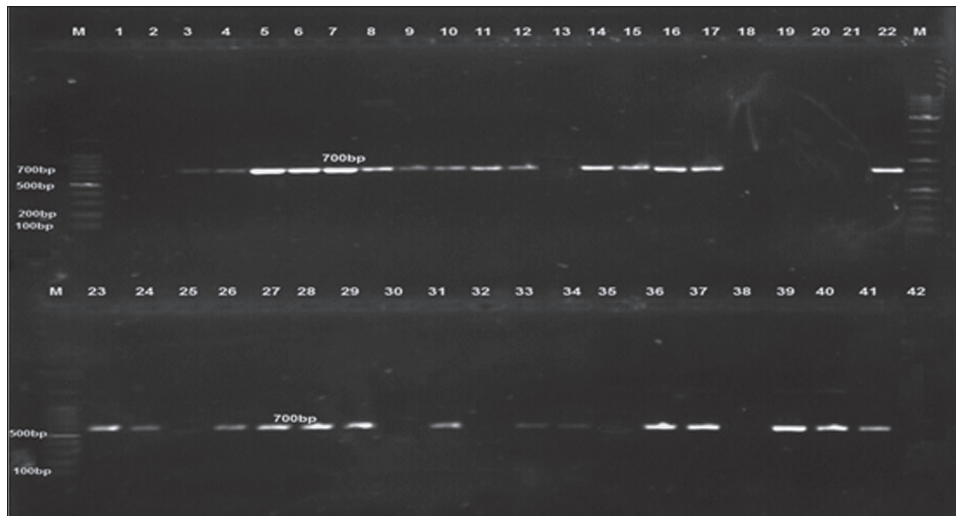
This study also revealed an infection rate with B19 of 16.5% (B19-IgM +ve) among women of childbearing age. Of the three different age groups illustrated in Table 2, IgM seropositivity showed an increase with age, starting from 8.1% among 15–25 years old to 18.6% among the 26–35-year-old, to reach the highest level of 36.4% for the 36–45-year-old group. These results were, however, not

statistically significant. The prevalence of B19-IgM among aborted women (32.5%) was higher than non-aborted (3.9%). The Pearson Chi-square test and the *P*-value indicate a significant correlation between B19-IgM and abortion status at a *P*-value below 0.01 at 95% CI. The prevalence of B19-IgM among were as follows: educated women (33.3%), unemployed (22.2%), and overcrowding families (31.1%), which were higher compared to non-educated (0.0%), employed (8.1%), and those living in non-crowded living arrangements (2.2%). The values of Chi-squared ( $\chi^2$ ) and *P*-values (*P*) indicate that the  $\chi^2$  figures for B19-IgM were significant both for education and residential status, with a *P*-value below 0.01 at a 95% CI. There is, however, no significant correlation between occupation status and the seroprevalence of B19-IgM [Table 2].

In order to confirm the infection with B19-V at the molecular level in the region, the sera from the IgG B19

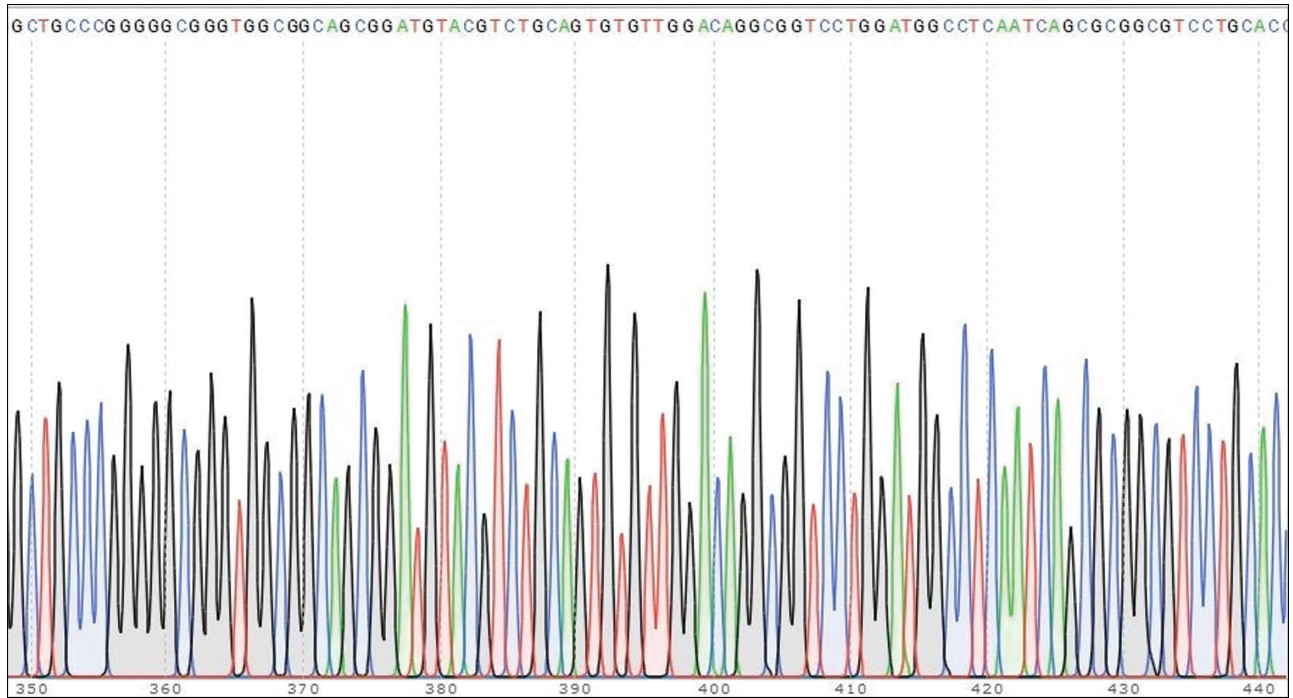
**Table 2: Seroprevalence of B19-V IgM among study population in regard to age, abortion, education, employability, and residential status**

	Age groups				Abortion status		Education status		Occupation status		Residential status	
	Total	15–25 years old	26–35 years old	36–45 years old	Not aborted	Aborted	Uneducated	Educated	Unemployed	Employed	Not crowded	crowded
<b>B19-V IgM –ve</b>	76	34	35	7	49	27	46	30	42	34	45	31
	83.5%	91.9%	81.4%	63.6%	96.1%	67.5%	100.0%	66.7%	77.8%	91.9%	97.8%	68.9%
<b>B19-V IgM +ve</b>	15	3	8	4	2	13	0	15	12	3	1	14
	16.5%	8.1%	18.6%	36.4%	3.9%	32.5%	0.0%	33.3%	22.2%	8.1%	2.2%	31.1%
<b>Pearson Chi-square</b>		5.18			13.300		18.360		3.17		13.386	
<b>P value</b>		0.075			<0.01		<0.01		0.070		<0.01	

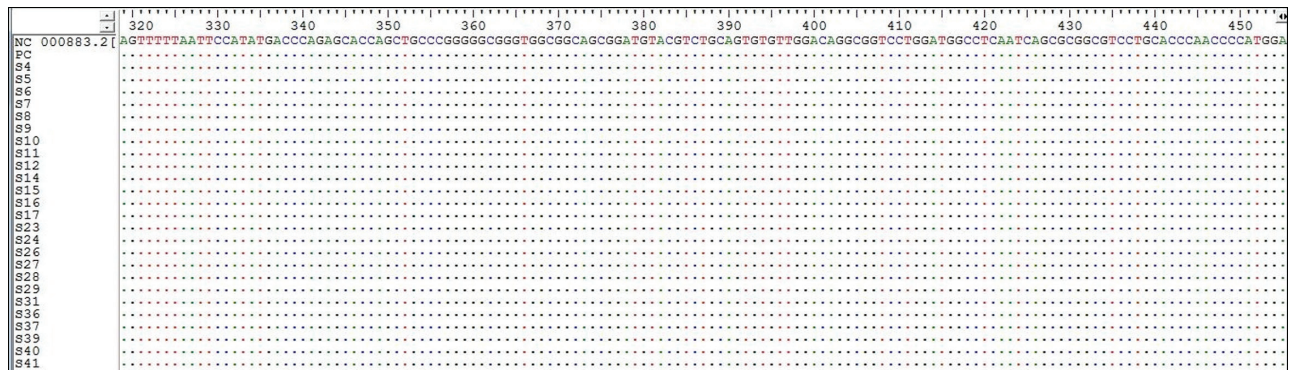


**Figure 1:** About 1.5% agarose gel electrophoresis (72 V/80 min) of PCR to amplify 700bp partial sequence from VP1 gene of 1–42 represented samples; M deoxyribonucleic acid marker size (100bp), PC (positive control) (sample no 22), NC (negative control) (sample no 42), all of the rest represent those tested positive for PCR (patients' samples)





**Figure 2:** The pattern of deoxyribonucleic acid (DNA) chromatogram for DNA specimens of partial sequence of the 700 bp amplicon of the VP1 gene



**Figure 3:** Partial Multiple deoxyribonucleic acid alignment of the 700 bp of the VP1 gene of human parvovirus B19

positively tested women ( $n = 40$ ) were subjected to PCR targeting a partial sequence of the VP1 gene. Positive amplicons (700bp) were detected from 27 samples of these women, as illustrated in Figure 1.

Upon sending the PCR products to Macrogen, Korea, for nucleotide sequencing, chromatograms derived from patients and positive control were obtained, as shown in Figure 2.

All nucleotide sequences obtained from patients and positive control were aligned using the DNA Star BioEdit program and found to be 100% identical among each other and to the reference “Human Parvovirus B19 (NC: 00088.3).” The numbers S4–S41 represent those 25 patients tested positive via PCR while PC represents the positive control [Figure 3].

Nucleotide sequences of the VP1 gene for all 26 positive PCR products (resembling 25 patients and one from PC)

were 100% identical among each other and to the reference “Human Parvovirus B19 (NC: 00088.3).” Dots indicate identical letters to the reference sequence (NC: 00088.3). The alignment was performed according to EditSeq DNA STAR software.

The obtained nucleotide sequence of the partial gene sequence of VP1 (700bp) was subjected to blast N in NCBI and found to be 100% identical to the human parvovirus viral protein (VPI) gene (ID\_000883.2) [Figure 4].

### DISCUSSION

Globally, according to recently published reports, the seroprevalence of B19-V among pregnant women in Europe is around 60%; in Italy, for instance, it is 69.5%.<sup>[23]</sup> This is higher than that detected among pregnant women

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**Human parvovirus B19, complete genome**

Sequence ID: [NC\\_000883.2](#) Length: 5596 Number of Matches: 1

[See 1 more title\(s\)](#) [See all Identical Proteins\(IPG\)](#)

Range 1: 3114 to 3818 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1303 bits(705)	0.0	705/705(100%)	0/705(0%)	Plus/Plus
Query 1	GAATAAATCCATATACTCATTGGACTGTAGCAGATGAAGAGCTTTTaaaaaatataaaaa	60		
Sbjct 3114	GAATAAATCCATATACTCATTGGACTGTAGCAGATGAAGAGCTTTTAAAAAATATAAAAA	3173		
Query 61	aTGAAGACTGGGTTTCAAGCACAAAGTAGTAAAAGACTACTTTACTTTAAAAGGTGCAGCTG	120		
Sbjct 3174	ATGAAGACTGGGTTTCAAGCACAAAGTAGTAAAAGACTACTTTACTTTAAAAGGTGCAGCTG	3233		
Query 121	CCCCGTGGGCCATTTTCAAGGAAGTTTGCCGGAAGTTCGCCCTTACAACGCCCTCAGAAA	180		
Sbjct 3234	CCCCGTGGGCCATTTTCAAGGAAGTTTGCCGGAAGTTCGCCCTTACAACGCCCTCAGAAA	3293		
Query 181	AATACCCAAGCATGACTTCAGTTAATCTGCAGAAAGCCAGCACTGGTGCAGGAgggggg	240		
Sbjct 3294	AATACCCAAGCATGACTTCAGTTAATCTGCAGAAAGCCAGCACTGGTGCAGGAgggggg	3353		
Query 241	gCAGTAATCCTGTCAAAGCATGTGGAGTGAGGGGGCCACTTTTAGTGCCAACCTGTGA	300		
Sbjct 3354	GCAGTAATCCTGTCAAAGCATGTGGAGTGAGGGGGCCACTTTTAGTGCCAACCTGTGA	3413		
Query 301	CTTGACATTTTCTAGACAGTTTTTAATTCATATGACCCAGAGCACCATTATAAGGTGT	360		
Sbjct 3414	CTTGACATTTTCTAGACAGTTTTTAATTCATATGACCCAGAGCACCATTATAAGGTGT	3473		
Query 361	TTTCTCCCGCAGCAAGTAGCTGCCACAATGCCAGTGGAAAGGAGGCAAGGTTTGCACCA	420		
Sbjct 3474	TTTCTCCCGCAGCAAGTAGCTGCCACAATGCCAGTGGAAAGGAGGCAAGGTTTGCACCA	3533		
Query 421	TTAGTCCCAATAATGGGATACTCAACCCCATGGAGATATTTAGATTTTAAATGCTTTAACT	480		
Sbjct 3534	TTAGTCCCAATAATGGGATACTCAACCCCATGGAGATATTTAGATTTTAAATGCTTTAACT	3593		
Query 481	TATTTTTTTCACCTTTAGAGTTTTCAGCACTTAATTGAAAAATATGGAAAGTATAGCTCCTG	540		
Sbjct 3594	TATTTTTTTCACCTTTAGAGTTTTCAGCACTTAATTGAAAAATATGGAAAGTATAGCTCCTG	3653		
Query 541	ATGCTTTAACTGTAACCATATCAGAAATTGCTGTTAAGGATGTTACAGACAAAAGTGGAg	600		
Sbjct 3654	ATGCTTTAACTGTAACCATATCAGAAATTGCTGTTAAGGATGTTACAGACAAAAGTGGAg	3713		
Query 601	ggggggTGCAGGTTACTGACAGCACTACAGGGCGCCTATGCATGTTAGTAGACCATGAAT	660		
Sbjct 3714	GGGGGGTGCAGGTTACTGACAGCACTACAGGGCGCCTATGCATGTTAGTAGACCATGAAT	3773		
Query 661	ACAAGTACCCATATGTGTTAGGGCAAGGTCAAGATACTTTAGCCC 705			
Sbjct 3774	ACAAGTACCCATATGTGTTAGGGCAAGGTCAAGATACTTTAGCCC 3818			

**Figure 4:** BLASTN alignment results of 700 bp partial gene sequence of viral protein (VP1) gene indicate 100% similarity to human parvovirus VP1 gene (ID\_000883.2)

in Africa (20%),<sup>[24]</sup> and among pregnant women in the Middle East, such as Iran, it is 54%.<sup>[23]</sup>

Previous research conducted in several parts of Iraq has found high seroprevalence rates of 93% in Kirkuk among women with BOH,<sup>[16]</sup> lower prevalence rates were reported in Erbil (40%),<sup>[17]</sup> and for ThiQar (38%)<sup>[18]</sup> in women with spontaneous abortion. Within this study, the overall seroprevalence of B19-V was 44%; this study found B19-V seroprevalence with the lowest rates among the youngest group (15–26) years old and increasing with age. The susceptibility rate of 66% identified in this study is within the national average. A significantly considerable link between the infection with abortion and BOH (control group) was also shown by our findings. Our study also revealed greater seroprevalence rates in overcrowded households, unemployed, educated, and aborted populations. This study found 44.0% seroprevalence of B19-IgG antibodies among the study population, which

is in alignment with those in ThiQar (38%),<sup>[18]</sup> Erbil (40%),<sup>[17]</sup> and considered lower than that reported for Kirkuk (93%).<sup>[16]</sup> Our findings are also comparable to that documented in the surrounding regions as in Iran (40%)<sup>[12]</sup> and Makkah, Saudi Arabia (46.6%).<sup>[25]</sup> The findings of this study are also in alignment with a previous study from Erbil, Iraq, which also documented a 40% seroprevalence rate of B19 IgG among women of childbearing age.<sup>[17]</sup> This study also found the seroprevalence of B19-IgG increased with age. There was also a higher seroprevalence of IgG among the aborted women (study group) (80%) and among those living in overcrowded situations (73.3%); our findings are also in accordance with the previous report from Kirkuk.<sup>[16]</sup>

The rising of B19 IgG seroprevalence with age among women in this study is consistent with findings from a previous study, which reported a rise from 5.8% in women under 20 years of age to a peak of 32.4% in those aged



between 30 and 40 years of age. These rates, however, were lower than that documented in our study: 37.8% for those aged 15–25 years old, 41.9% for 26–35 years old, and approximately 72.7% for the geriatric group. Similar findings have come out from studies in other countries such as the USA, Australia, and Iran.<sup>[26,27]</sup> In the current study, B19-IgG seroprevalence continued to increase among the 36–45 year olds, reaching the highest at 72.7%. Indeed, this finding is comparable with other studies that have reported levels of approximately 60%–80% B19-IgG seropositivity among the geriatric age groups.<sup>[26]</sup> Seropositivity of IgG is believed to confer lifelong immunity. The finding of a seropositivity rate of 44.0% to B19-V infection among recruited women in the Al-Hamza district in Iraq in this study is noteworthy. This finding shows a difference of approximately 4% from Ahga *et al.* (2020),<sup>[28]</sup> who found a seropositivity rate of approximately 40% in Erbil, Iraq. This descriptive case–control study reported a higher seroprevalence of IgM among the aborted (32.5%), educated (33.3%), unemployed (22.2%), and overcrowded (31.1%). The role of sociodemographic factors in the epidemiology of B19 is well documented.<sup>[29]</sup> The increasing levels of B19 IgM antibodies increased from 8.1% among the youngest group (15–25 years) to 18.6% among the second age group (26–35 years) to reach the highest levels at 36.4% among the older group (36–45 years) is aligned with the high levels of current infections among the aborted women. This rise of IgM seroprevalence from those below 20 years of age to a peak among 36–45 years old age group is aligned with other studies.<sup>[26–30]</sup>

The high seroprevalence of B19-IgM among those living in overcrowded households and among homemakers (unemployed) aligns with the results of similar studies. Women whose social activities expose them to young children are noted in studies to be at higher risk of B19 infections. Women in their late 20s or 30s are more likely to be multiparous, have children of their own, engage in school runs, and engage with children from other families through the social activities of their children.<sup>[30]</sup> The interaction of large numbers of seronegative children within these contexts creates infection pools of active shedding episodes, which can persist for prolonged periods.<sup>[31]</sup> Similar to other viral infections such as CMV,<sup>[32]</sup> B19 is usually transmitted through body secretions, for example, nasal secretions, and women involved in cleaning and other sanitary activities. For similar reasons, women who work in after-school clubs, day-care centers, or nurseries have also been documented to be at higher risk for such viruses. Women with para-one are at three times greater risk than the nulliparous woman, while the risk of B19 infection increases to 7–8 times for those with 3 or more children at home.<sup>[33]</sup>

Fetal loss from congenital B19 infection is the most common in the first 20 weeks of pregnancy, with fetal

death occurring approximately 28–42 days post B19-V infection.<sup>[9,34]</sup> In the current study, among aborted women in Babil, Iraq, 80.0% IgG and 32.5% IgM seropositivity were detected. Our data revealed a highly significant correlation between the seroprevalence of B19-IgG and IgM with abortion ( $P < 0.01$ ). This is comparable to other studies in the region; for example, Khameneh *et al.* reported an 88.0% IgG seroprevalence among aborted women in Iran.<sup>[35]</sup> The high seroprevalence of IgG and IgM of B19-V found in this study among those individuals with BOH supports the advice to screen pregnant women on a regular basis, particularly if they may have been exposed to the virus through interactions with their own or other families' children.

Based on sequencing analysis of the partial gene sequence of VP1 from B19-V, the nucleotide sequences were 100% identical to the human parvovirus VP1 gene (ID\_000883.2), and this, in turn, confirms the infection with B19-V among Iraqi women at childbearing age. A previous study from Iran<sup>[12]</sup> has recorded B19-V, Genotype 1, subtype 1a, as a circulating B19-V in the region. Further studies molecularly characterizing B19-V and determining the genotype and subtype are needed in the region.

Locally, previous studies conducted found an infection rate with B19-V of 30.4% and 31% among women with adverse pregnancy outcomes.<sup>[36,37]</sup> In the current study, the main goal of the PCR tool was to amplify a partial sequence of the VP1 gene to establish the infection with B19-V among the Iraqi population, and it was not to be correlated with the serological results. The 27 patients' samples showed positive PCR amplicons out of the  $n = 40$  positively tested women (67.5%) for B19-V IgG do not represent infection rate simply because PCR was performed only for samples tested positive for B19-IgG and not for the entire study population. The explanation of B19 viral DNA detection in our patient group remains ambiguous basically because of the persistence of low-level viremia post-viral infection.<sup>[24,25]</sup> This might explain the less than 100% detection rate via PCR from those women who tested positive for B19-V IgG through ELISA. Case–control studies are, therefore, useful in estimating the association between DNA detection of B19V and the disease. Using internal positive controls was of significant value to examine the existence of inhibitory substances during PCR and sequencing procedures.<sup>[38]</sup>

## CONCLUSION

This is the first study into the molecular and serological detection of B19-V among women with BOD in the Al-Hamza district of Hilla, Iraq. Based on the results of this study, B19-V is endemic in Babil, Iraq. The study found about 66% of women with BOH were susceptible to B19-V infection, which indicates a need for screening of all pregnancies for B19 immunity at the earliest

possible opportunity. Such screening will help to identify susceptibility and evidence of any seroconversion at a later stage of the pregnancy. Lifelong immunity is believed to result from B19 infection demonstrated by IgG prevalence. To conclude, this paper highlights the need for B19-V seroprevalence and molecular data to be further researched and explored in local populations. This will subsequently inform proactive intervention measures on regional levels.

### Author contribution

Maha Diekan Abbas collected the data and wrote the manuscript, Haider Turkey Mousa Al-Mousawi revised the manuscript, Nadhim Mushtaq Hashim Al-Bdere designed the experiment and assisted in molecular analyses, and Rasha M.A. Al-Humairi conducted the statistical analyses.

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### Conflicts of interest

There is no conflict of interest.

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