

Environmental Monitoring of Parvovirus B19 in the Kidney Transplantation Ward of a Chinese Teaching Hospital

Fang Wang^{1,*}, Qing Zhan^{1,*}, Shi-Ping Yu², Hai-Ting Feng¹, Ping Hu², Zi-Feng Zhong¹, Ting-Ting Qu^{1,3}

¹Infection Control Department, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, 310003, People's Republic of China;

²Kidney Disease Center, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, 310003, People's Republic of China; ³State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, 310003, People's Republic of China

*These authors contributed equally to this work

Correspondence: Ting-Ting Qu; Zi-Feng Zhong, Email qutingting@zju.edu.cn; 1182009@zju.edu.cn

Purpose: Parvovirus B19 (B19V) infection is a viral threat after kidney transplantation. It is mainly transmitted by close-contact inhalation of aerosolized viral particles. The risk of nosocomial spread of B19V in the transplantation ward is quite high. This study aimed to evaluate the quality of routine disinfection and the effectiveness of isolation measures in the wards of B19V-infected kidney transplant recipients.

Patients and Methods: Throat swab samples of 19 kidney transplant recipients admitted to the isolation ward and three healthcare workers (HCWs) were collected for viral DNA detection. Routine disinfection procedures were performed twice a day in general and B19V isolation wards. Environmental surface and air samples were collected for viral DNA detection before and after disinfection.

Results: A total of four patients were diagnosed with B19V infection and transferred to the B19V isolation ward, of which only two had positive throat swab samples. The other 15 patients and all HCWs tested negative for B19V. A total of 88 environmental surface and air samples were collected. Eight of the environmental samples collected in the B19V isolation ward before disinfection tested positive for B19V, while one sample tested positive after disinfection. In the general wards, all environmental samples collected before disinfection tested negative for B19V. All 24 samples collected from ambient air, whether in B19V isolation or general wards, before or after disinfection, tested negative for B19V.

Conclusion: Existing methods of routine or terminal disinfection for air and object surfaces were effective in eliminating B19V from object surfaces and ambient air in the isolation and general wards. Material surfaces that are exposed to high frequency and easily contaminated by blood, body fluids, and indoor air were the focus of cleaning and disinfection. Nosocomial cross-infection of other immunocompromised patients and HCWs can be avoided if appropriate prevention and control measures are taken.

Keywords: parvovirus B19, B19V, kidney transplantation ward, isolation, disinfection

Introduction

Parvovirus B19 (B19V), a member of the large *Parvoviridae* family and *Erythroparvovirus* genus, is a small, non-enveloped, single-stranded DNA (ssDNA) virus that infects only humans.¹ It is a common community-acquired respiratory pathogen without ethnic, socioeconomic, gender, age, or geographic boundaries.² Infectivity is temperature-dependent, with the infection being most frequent in the winter and spring.³ B19V is mainly transmitted in close contact by the inhalation of viral particles in aerosol (ie, respiratory spread transmission).⁴ Transmission may also occur through blood-donor transfusion and transplantation, mostly during the viremia that precedes the seroconversion period, as well as vertically, from mother to fetus.⁵ B19V is typically acquired during childhood, and at lower rates throughout adulthood, such that between 70% and 85% of adults show serologic evidence of past infection. Infection with B19V causes several clinical syndromes (fifth

disease, transient aplastic crisis, pure red cell aplasia, hydrops fetalis, glomerulopathy, and anemia in end-stage kidney disease) and may contribute to other illnesses.⁶ Transient aplastic crisis because of B19V infection is of particular concern in patients with either decreased red blood cell production or increased turnover.⁴

Owing to their immunosuppressed status, kidney transplant recipients are particularly prone to acquire B19V infection, and it is a new viral threat in post-kidney transplantation.⁷ One of the most serious common diseases in immunosuppressed allograft recipients is B19V-associated pure red cell aplasia (PVB19-PRCA).⁸ Specific antiviral drugs or therapy are not available to treat B19V infection, and there is no effective vaccine for prevention. The treatment approach for infection depends on host factors such as immune status, underlying conditions, and manifestations of infection. Intravenous immunoglobulins (IVIG) and a reduction of immunosuppression make up the cornerstone of therapeutic management of B19V infection post-kidney transplantation.^{9,10} Patients with transient aplastic crisis may need supportive therapy with blood transfusions until the neutralizing antibody response can clear the virus and hematopoiesis is restored.¹¹ B19V infection can persist and relapse frequently in individuals with compromised immune systems, which may affect patient prognosis and increase treatment costs.⁴

Due to the relative ease of spread of the virus among both hospital staff and patients, the risk of nosocomial spread of the disease, acquired from close contact or environmental surfaces, is quite high, with reported attack rates of 50%.^{12,13} Control measures such as prompt disinfection, hand hygiene, ward closure, and B19V education are likely crucial for containing transmission.¹⁴ To avoid the contagion, standard and droplet precautions and isolation should be implemented. Although many cases of B19V infection in kidney transplant recipients have been reported, in the last few decades there have been few relevant evaluations concerning environmental monitoring for B19V during their hospitalization.^{15–17} In order to identify potential risk factors for B19V infection, and ensure effective isolation of infected patients and a safe living environment for other patients with compromised immune systems after transplantation, we performed a thorough environmental surveillance, including air and surface sampling; meanwhile, we continued to sample the respiratory tract of medical staff and patients to evaluate the quality of routine disinfection and effectiveness of isolation measures in the wards of B19V-infected kidney transplant recipients.

Materials and Methods

Study Design

This study was conducted in the protective isolation ward for kidney transplantation. After kidney transplantation, patients were admitted to this ward and would transfer to the general ward or discharged directly after their condition stabilized in 1–2 weeks. There were three beds in each ward and one public restroom. The patients were appropriately restricted to activities inside the ward. Patients diagnosed with B19V infection were immediately isolated in the same room, with a bed spacing of ≥ 1.1 m. The floor plan of the ward is shown in [Figure 1](#). A special buffer zone was set up in this ward, where the staff would change to special shoes, work clothes, surgical caps, surgical masks, and an isolation gown before entering the ward. The staff strictly performed hand hygiene before and after contact, treatment, or care of patients; in addition, gloves were worn when necessary.

The B19V epidemic season was selected as the research time, and the B19V isolation ward where the infected patients were located, and the adjacent ward managed by the same medical care group were selected as the research objects. From June 1, 2020 to June 29, 2020, a total of 19 patients were admitted to our study area, of which 15 were free from B19V infection and were transferred to general wards. Four patients tested positive for B19V about one week after transplantation, confirmed at two different times, and were moved to the B19V isolation ward. The time in the hospital of the four confirmed B19V-positive patients was slightly longer than that of the 15 B19V-negative patients in the general ward. It was confirmed that every two patients were received the same donor and were non-living transplant recipients. All donors were tested for B19V DNA, and one donor was positive. Daily diagnosis and treatment and the normal entry and exit of patients were not affected during the study period.



Figure 1 The floor plan of the kidney transplantation ward.

Disinfection

The entire ward was cleaned and disinfected twice a day by using disposable dry towels or clean floor towels soaked with 500 mg/L of chlorine-containing disinfectant to wipe or mop the surface of objects and the floor and waiting for 30 min. Routine cleaning and disinfecting of B19V isolation wards were done after all other wards. The medical equipment utilized was wiped after each use with Gama Clinell universal wipes containing 0.765–0.935% active ingredient. Any place suspected of being polluted or with visible pollution, including bed railings, bedside tables, furniture, door handles, floors or other articles, were completely cleaned and disinfected at any time. Continuously running plasma air disinfection machines were used for air disinfection in B19V isolation wards. The return air filter of the air purification device was cleaned weekly and at the end of the terminal disinfection.

After a patient was discharged or transferred, terminal disinfection of the bed unit was performed in the general ward and the B19V isolation ward. The concentration of chlorine disinfectant was increased to 2000 mg/L for the terminal disinfection in the isolation ward; after 30 min of disinfection the ward was wiped with clean water and a mobile ultraviolet lamp was used for air disinfection for more than 1 hour before ventilation.

Collection of Environmental and Air Samples for Detection

A virus sampling tube and accompanying swab (yocon[®], Beijing, China) were used to collect throat swab samples from patients with confirmed B19V, patients in the general wards of the same medical care group, and the medical staff. The sampling sites are marked in Figure 1; the blue dots represent environmental surface sampling sites and the yellow dots represent air sampling sites. Surfaces deemed to be high-contact sites were sampled using swabs wetted with universal transport medium (UTM). Environmental surface sampling was performed on the high-frequency contact surfaces, including bed rails, bedside tables, toilet washbasins, and the flush button of the toilet bowl in the patient's hospital room, and the return air outlet surface of the air purification device. The surface and throat swab samples were placed in UTM containing Hanks' balanced salt solution, amino acids, glycerin, and other ingredients as described by Moore et al, pending inspection.¹⁸ Three air sampling sites were selected and marked with yellow dots as seen in Figure 1. A nutrient

agar medium with a diameter of 9 cm was used to collect air samples by the sedimentation method.¹⁹ This agar medium containing the air samples was transferred to a 15 mL centrifuge tube, 4 mL of double-distilled water (ddH₂O) was added, and the agar was dissolved in a 37°C water bath for 10 min, pending inspection.

DNA Extraction and Real-Time Quantitative Reverse Transcription-PCR

Genomic DNA (gDNA) was extracted from the samples by heat treatment according to the manufacturer's instructions. Commercial human parvovirus B19 real-time polymerase chain reaction (PCR) kit (Liferiver™, Shanghai ZJ Bio-Tech Co., Ltd.) was used for the detection and quantification of B19V DNA in the samples. The lowest detection limit for this test was 1×10^3 copies/mL and the linear range of detection ranged from 2×10^3 to 1×10^8 copies/mL. A sample with a viral load corresponding to $\geq 1 \times 10^3$ copies/mL was considered to be positive.

Results

Throughout the study period four patients with confirmed B19V were hospitalized and lived in the B19V isolation ward at two different times. It was confirmed that every two patients were received the same donor and were non-living transplant recipients. One donor was positive for B19V DNA at screening. The remaining 15 patients lived in general wards with shorter hospital stays. Twenty-two respiratory samples of patients and medical staff were collected. Among the four patients with confirmed B19V, two respiratory throat swab samples tested positive for B19V but the detection value of the remaining two did not reach the positive threshold. All throat swab samples from three medical staff involved in the study and the 15 patients in the general wards tested negative for B19V (Table 1).

The sampling sites with high-frequency contact were sampled before and after disinfection and are marked in Figure 1. A total of 88 environmental surface and air samples were collected, including 44 from the B19V isolation ward and 44 from the non-isolation ward. Among all the samples, 22 samples came from the wards before disinfection, including 16 from environmental material surfaces and 6 from the air. After disinfection, samples were collected at three different time periods (3 h, 5 h, and 14 h after disinfection), for a total of 48 environmental surface samples and 18 air samples (Table 2).

Seven of the environmental material surface samples tested negative for B19V before disinfection in the general ward. Eight of the environmental samples tested positive for B19V before disinfection in the B19V isolation ward, and originated from the bed bar, bedside table, toilet washbasin, and the return air outlet of the air purification and disinfection device; meanwhile, one sample from the flush button of the toilet bowl tested negative (Table 2). After disinfection, one sample from an environmental material surface tested positive for B19V in the isolation ward, originating from the washbasin of the patient's bathroom 3 h after disinfection, while the remaining 47 samples tested negative (Table 3). We confirmed that a B19V confirmed patient spit out mouthwash into the disinfected washbasin and failed to rinse it in time. The difference in the number of B19V positive surface samples detected before and after disinfection in B19V isolation wards was statistically significant ($p < 0.01$). Air sampling sites are marked with yellow dots in Figure 1 and three locations were selected. A total of 24 samples were collected, including 12 in the general ward and 12 in the B19V isolation ward. Each ward included 3 samples before disinfection and 9 after disinfection. All 24 samples collected from ambient air, tested negative for B19V (Table 4).

Table 1 B19V RT-PCR^a Results of Respiratory Tract Samples from Patients and Healthcare Workers

Source of Respiratory Tract Samples	B19V + ^b /Total ^c	Results ^d
B19V-confirmed patients in isolation wards	2/4	7.4×10^4 , 1.4×10^5
Patients in general wards	0/15	/
Doctors in charge	0/2	/
Primary nurse	0/1	/

Abbreviations: ^aRT-PCR, reverse transcription polymerase chain reaction; ^bB19V+, number of positive samples; ^cTotal, total number of samples; ^dRT-PCR, results (copy number/mL).

Table 2 B19V RT-PCR^a Results of Environmental Surface Samples Before Disinfection

Areas	Sampling Sites	B19V + ^b /Total ^c	Results ^d
B19V isolation wards	Bedside tables	2/2	4.5*10 ³ , 1.0*10 ⁴
	Bed rails	2/2	1.0*10 ⁴ , 1.8*10 ³
	Toilet washbasins	1/1	1.4*10 ³
	Flush button of the toilet bowl	0/1	
	Return air outlet surface of the air purification device	3/3	3.2*10 ³ , 3.5*10 ³ , 2.7*10 ³
General wards	Bedside tables	0/1	/
	Bed rails	0/1	/
	Toilet washbasins	0/1	/
	Flush button of the toilet bowl	0/1	/
	Return air outlet surface of the air purification device	0/3	/

Abbreviations: ^aRT-PCR, reverse transcription polymerase chain reaction; ^bB19V+, number of positive samples; ^cTotal, total number of samples; ^dRT-PCR, results (copy number/mL).

Table 3 B19V RT-PCR^a Results of Environmental Surface Samples After Disinfection

Areas	Sampling Sites	3 h Later	5 h Later	14 h Later	Results ^d
		B19V + ^b /Total ^c			
B19V isolation wards	Bedside tables	0/1	0/2	0/1	/
	Bed rails	0/1	0/2	0/1	/
	Toilet washbasins	1/1	0/1	0/1	1.7*10 ³
	Flush button of the toilet bowl	0/1	0/1	0/1	/
	Return air outlet surface of the air purification device	0/3	0/3	0/3	/
General wards	Bedside tables	0/2	0/1	0/2	/
	Bed rails	0/2	0/1	0/2	/
	Toilet washbasins	0/1	0/1	0/1	/
	Flush button of the toilet bowl	0/1	0/1	0/1	/
	Return air outlet surface of the air purification device	0/3	0/3	0/3	/

Abbreviations: ^aRT-PCR, reverse transcription polymerase chain reaction; ^bB19V+, number of positive samples; ^cTotal, total number of samples; ^dRT-PCR, results (copy number/mL).

Table 4 B19V RT-PCR^a Results of Ambient Air Samples Before and After Disinfection

Sampling Sites	Before Disinfection	3 h Later	5 h Later	14 h Later	Results ^d
	B19V + ^b /Total ^c				
B19V isolation wards	0/3	0/3	0/3	0/3	/
General wards	0/3	0/3	0/3	0/3	/

Abbreviations: ^aRT-PCR, reverse transcription polymerase chain reaction; ^bB19V+, number of positive samples; ^cTotal, total number of samples; ^dRT-PCR, results (copy number/mL).

Discussion

B19V infection is common worldwide, showing regional epidemiological differences with generally over one-half of the adult population having been exposed.⁴ The first case of B19V infection in a kidney transplant recipient (KTR) was reported in 1986.¹⁵ Among solid-organ transplant recipients, most seronegative KTRs of B19V-positive organs became infected within 1 month, and could develop persistent refractory anemia with reticulocytopenia, which posed a potentially serious threat to the allograft and recipient survival.²⁰ This is similar to our research, in which most kidney transplant patients became infected with B19V within 1 week. In cases where the donor had low-level B19 DNAemia, the recipients were less likely to have obvious clinical manifestations, and the B19V could be quickly cleared even if the recipient was infected; however, high levels of B19 DNAemia may lead to graft failure.^{21,22} Previous studies at our

hospital also showed that higher B19V viral loads in recipients within 7 days after transplantation were positively correlated with donor-derived B19V infection in deceased-donor kidney transplantation.²³

Nonenveloped viruses of the *Parvoviruses* family can resist harsh environmental conditions, surviving on surfaces for long periods with the possibility of being transmitted to susceptible hosts. Parvoviruses are also tolerant to several mild disinfectants but sensitive to halogens, such as chlorine-releasing agents, which are often used for decontamination of ward floors and facility surfaces due to their broad spectrum of activity.^{24,25} According to some studies, parvoviruses can cause nosocomial cross-infection directly through contact with respiratory droplets or contaminated surfaces, and the parvovirus attack rates among patients or HCWs might not be as low as once thought.^{12,13,26} Therefore, strict implementation of isolation measures, personal protection, and environmental cleaning and disinfection is crucial.²⁷

In our study, among the four isolated patients with B19V confirmed with blood samples by the laboratory, only two tested positive for B19V through throat swab samples; meanwhile, the detection value of the remaining two patients did not reach the positive threshold. Patients can cause B19V positivity in the surrounding environment through the respiratory tract and daily contact. There was B19V contamination on the high-frequency contact surfaces of the B19V isolation ward before disinfection, such as bed rails, bedside tables, toilets, sinks, and the return air outlet surface of the air purification device; even without visible contamination they were all positively detected. This result also confirms that isolation in a single room or isolation of the same disease is necessary, otherwise transmission of B19V and cross infection among other patients after transplantation and HCWs could occur through the contaminated environment. We believe that it is very important to increase patient health education to improve hand hygiene and reduce environmental pollution via the respiratory tract and daily contact. Additionally, high-frequency contact surfaces and surfaces that are easily contaminated by blood and body fluids should be the focus of daily surface cleaning and disinfection, while effective terminal disinfection can avoid persistent infections in newly admitted patients. This study shows that the positive detection of B19V DNA on environmental surfaces can be reduced by correct disinfection.

Considering the stimulating effect of chlorine-containing disinfectants on the respiratory tract, a concentration of 500 mg/L was used for routine disinfection at 6 am and 3 pm every day. The concentration of chlorine disinfectant was increased to 2000 mg/L during the terminal disinfection in the B19V isolation ward. In our results, all surface samples collected in the B19V isolation ward after disinfection were negative except for the washbasin, which tested positive for B19V. We confirmed that a B19V-positive patient vomited mouthwash into the disinfected washbasin and failed to rinse it in time. We communicated with patients and told them to wash the basin with running water immediately after each dumping of contaminated liquid, and there were no B19V positive samples detected after this improvement. Similarly, patients admitted to the ward after terminal disinfection did not develop B19V infection, indicating that the cleaning and disinfection methods used were effective. The frequency of cleaning and disinfection should be increased for the surface of objects easily contaminated by blood and body fluids. Before disinfection, B19V in the air did not reach the positive threshold mainly due to the single unit laminar air flow system in all isolation wards. However, the return air outlet surface of the air purification device was positive for B19V DNA detection before disinfection, which indicates that B19V could be attached to the environmental surfaces through air transmission. B19V is between 23 and 28 nm in size and thus a laminar flow system cannot completely purify the air, requiring plasma air disinfection machines running continuously.^{4,28}

In addition, we implemented various disinfection and isolation measures for infected patients. Healthcare workers and cleaning staff wore full personal protective equipment (PPE) when entering and leaving the isolation ward, properly put on and took off PPE, and increased hand hygiene compliance per “My five moments for hand hygiene”.²⁹ Although the staff responsible for the diagnosis, treatment, care, and sanitation of the isolation and general wards were the same group, their respiratory tract samples and all environmental and air samples from the general ward tested negative. These results indicate that nosocomial cross-infection can be avoided if appropriate prevention and control measures are taken.

There are several limitations to this study. First, the positive samples were not cultured to prove their viability and infectivity. Second, the source of B19V infection in patients after kidney transplantation was not very clear. Among the four isolated patients with confirmed B19V in this study, it was confirmed that every two patients were received the same donor and were non-living transplant recipients. One donor screened positive for B19V DNA detection. Therefore, screening for B19V is recommended for kidney transplant donors with a long history of hospitalization, especially in the

intensive care unit, who may have low immunity, as well as implementation of early isolation observation and monitoring of such recipients. However, to the best of our knowledge, this is the first study to examine the environmental hygienic monitoring of parvovirus B19 in a kidney transplantation ward of a general hospital, although there have been many reports of hospital outbreaks of B19V.^{12,30,31} Our study provides an important evidence-based basis and reference value for providing targeted disinfection guidance and avoiding parvovirus cross-infection for immunosuppressed patients undergoing kidney transplantation.

In conclusion, existing methods of routine or terminal disinfection for air and object surfaces are effective. Material surfaces that are exposed to high frequency and easily contaminated by blood and body fluids and indoor air should be the focus of cleaning and disinfection. Ensuring adequate frequency of cleaning and disinfection every day and disinfection at any time is also very important to control pollution. Nosocomial cross-infection of other immunocompromised patients and staff can be avoided if appropriate prevention and control measures are taken.

Ethical Approval and Consent to Participate

Written informed consent was obtained from all the patients involved in this study. All kidneys were donated voluntarily with written informed consent during the upfront diagnosis and treatment process, which was carried out in accordance with the Istanbul Declaration. This study was approved by the local ethics committees of the First Affiliated Hospital, College of Medicine, Zhejiang University.

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Author Contributions

FW, SY, HF, PH collected samples and performed laboratory measurements. FW and QZ collated and analyzed the data. FW and QZ drafted the article. TQ and ZZ made substantial contributions to the study's conception and design and made a critical revision to the article. All the authors read and approved the final version of the manuscript. All authors contributed to data analysis, drafting, or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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