Review

Comprehensive analysis of parvovirus B19 infection in blood donors: epidemiological trend and implications for transfusion safety and management strategies in Italy

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Parvovirus B19 (B19V) presents a significant concern in the context of blood transfusion safety, given its potential for transmission through contaminated blood products, and the increased viral circulation recently reported across Europe. This study examines the recent epidemiological trends of B19V in Italy, where a notable increase in B19V-positive plasma units was observed during early 2024. While routine NAT testing for B19V in individual blood donations is not currently justified, the existing screening protocols for plasma intended for industrial fractionation are crucial to ensure the safety of plasma-derived medicinal products. However, this situation requires careful consideration of the optimal management of viremic donors and the implementation of targeted look back procedures to trace and monitor recipients of labile blood components. To address these issues, we propose an algorithm for managing both donors and recipients in cases of B19V positivity. These measures aim to balance recipient safety with minimising donor loss, while also addressing significant operational and ethical considerations within blood establishments.

Keywords: *parvovirus B19, transfusion safety, infectious diseases, epidemiological trend, management strategies.*

INTRODUCTION

Parvovirus B19 (B19V) is a small, non-enveloped, single-stranded DNA virus of the *Parvoviridae* family, known primarily as the cause of *erythema infectiosum*, or fifth disease, which mainly affects children[1]. While fifth disease typically presents as a mild rash illness with a "slapped cheek" appearance and may include mild fever and joint pain, B19V can lead to more severe complications in vulnerable populations, such as pregnant women, where it can cause fetal hydrops or miscarriage, and in individuals with weakened immune systems or chronic hemolytic disorders, leading to chronic anemia or aplastic crisis[1-5]. B19V's predilection for infecting erythroid progenitor cells in the bone marrow poses particular concerns for transfusions of blood and blood products, as the virus can be transmitted through contaminated blood components and cause serious complications in susceptible blood recipients[6-10].

To mitigate these risks, fractionation companies have long implemented B19V DNA screening on plasma as raw material for the production of plasma-derived medicinal

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products (PDMPs). This screening is crucial for ensuring the safety of final products by detecting high viremic plasma units, thereby preventing the accumulation of high viral loads in plasma pools, which could compromise viral inactivation processes and potentially transmit B19V to thousands of recipients[11-15]. However, B19V DNA testing for labile blood components is generally not performed routinely, as the risk of severe infection is low and most recipients are either immune or develop protective antibodies, reducing the likelihood of transmission through these products in conditions of normal viral circulation[7,16-21].

The need for vigilant monitoring of B19V within the donor population has intensified, especially in light of recent epidemiological data indicating a significant rise in B19V circulation across Europe. In May 2024, the European Centre for Disease Prevention and Control (ECDC) reported a notable increase in B19V positivity across nine EU/EEA countries, affecting pediatric populations and pregnant women. This surge has led to heightened scrutiny within the transfusion community regarding the safety of blood components[22-23].

In response, the ECDC conducted a survey in April 2024 to assess the performance of B19V screening tests and the prevalence of B19V infections within the donor population. Among the 18 responding countries, many do not routinely screen for B19V, yet several reported increased B19V reactivity in donations intended for industrial fractionation during early 2024 compared to the same period in 2023. This trend is concerning given B19V's high viral load during the acute phase, which increases the risk of transmission through blood products[22].

Preliminary data from Italy revealed a significant increase in B19V-positive plasma units intended for fractionation from late December 2023 through the first half of 2024. This underscores the urgent need for a thorough assessment of the implications of B19V infection on transfusion safety. The Italian National Blood Centre has been instrumental in coordinating this assessment, given the rising incidence of B19V detected by fractionation companies during this period.

The objective of this paper is to review recent epidemiological data on B19V infection within the Italian donor population and to draft precautionary recommendations for preventing transfusion-transmitted B19V infections, including criteria for the deferral of viremic donors and a look-back approach for patients who have received labile blood components from donors later found to be viremic. Additionally, this study aims to offer valuable insights to the broader European context, providing guidance to other national competent authorities facing similar increases in B19V infections.

PARVOVIRUS B19V INFECTION AND BLOOD TRANSFUSION

Evidence of transfusion transmission of B19V

The transmission of B19V through Substances of Human Origin (SoHO) has been documented in various contexts, including the transfusion of red blood cells, platelets, PDMPs, and the transplantation of hematopoietic stem cells (HSCs) and solid organs. Despite these documented routes, clinically significant cases of B19V infection resulting from transfusion appear to be rare in Europe. For example, only one case of transfusion-transmitted B19V was reported in the United Kingdom between 1996 and 2022. Similarly, Germany reported no cases from 1997 to 2017, even without routine B19V testing for blood donors[22]. In Italy, hemovigilance data from 2013 to 2023 also report no cases, with only one documented instance of B19V transmission *via* transfusion occurring in a patient with major thalassemia in the 1990s[10].

Studies have shown that B19V DNA can persist for months or even years due to the sensitivity of modern detection methods. During the acute phase, high concentrations of B19V DNA are detectable, with levels peaking during viremia. Following this stage, DNA concentrations typically decrease as neutralizing IgG antibodies are produced. Persistence of B19V DNA in tissues, such as liver, heart, and synovium, suggests that B19V DNA positivity in plasma units likely reflects the presence of non-infectious "naked" DNA strands rather than active virions. Most B19V-positive donations, especially those with low DNA concentrations, are therefore unlikely to pose an infectious risk to recipients[16-17,24].

Several studies suggest that transmissibility *via* transfusion is associated with DNA levels of 10^5 IU/mL or higher, while most blood products contain B19V DNA below this threshold. Additionally, donors with ongoing B19V infection typically have neutralizing antibodies, providing further protection for recipients[7,16-21]. However,

exceptions exist, as documented by a Japanese study where B19V transmission occurred through a red cell concentrate containing 5.1×10^3 IU/mL of B19V DNA, along with both IgG and IgM antibodies in the donor's plasma. In contrast, no B19V infection was observed after transfusion of 15 blood component units (eight red blood cell concentrates, four platelet concentrates, and three fresh frozen plasma samples) from donors with B19V DNA concentrations between 10^3 and 10^4 IU/mL in plasma[16].

Although IgM increase is usually expected during the acute phase of infection, it may not always be detectable at the peak of viremia, making anti-B19V IgM screening unreliable for identifying at-risk donations[16].

Risk assessment in transfusion recipients

Unlike HBV, HCV and HIV, B19V infections generally have a marginal public health impact in terms of transfusion-transmitted infections. However, the cumulative risk associated with PDMPs remains an issue of major concern, particularly due to the partial efficacy of viral inactivation methods against non-enveloped viruses. The ECDC conducted a risk assessment for B19V infection across four key population groups[22]:

- general population: the risk is low, as most infections result in mild childhood exanthematous disease, though complications can occur;
- pregnant women: the risk is assessed as low to moderate, particularly below 20 weeks gestation, due to uncertainties about viral circulation; serious outcomes, however, are rare;
- immunocompromised individuals, including transplant patients: the risk is moderate, as these patients may experience chronic anemia, pancytopenia, graft loss, or organ-invasive disease;
- individuals with chronic hematologic diseases (e.g., sickle cell anemia, thalassemia): the risk is also moderate, with B19V potentially causing transient aplastic crises.

Measures to mitigate the risk of transfusion transmission

Plasma units intended for fractionation are pooled into production batches and tested and manufactured as mandated by the European Pharmacopoeia. To mitigate the risks of B19V transmission through PDMPs, fractionation companies have also implemented B19V DNA testing in plasma mini-pools. This screening is specifically aimed at detecting donations with high viremia levels in mini-pools of plasma intended for industrial fractionation and was implemented following reports of B19V seroconversion in volunteers participating in a pharmacovigilance study on virus-inactivated with solvent/detergent plasma (SD plasma), generally due to high viral loads (B19V DNA >10^7 IU/mL)[11-13]. The introduction of B19V DNA testing on industrial mini-pools, conducted by pharmaceutical companies, allows for the detection and elimination of potentially infected plasma units before they enter the industrial pool. This screening is considered a control measure within the manufacturing process of PDMPs rather than a screening of individual donations. It is necessary to prevent the accumulation of viral loads exceeding 10^4 IU/mL in individual donations pooled together, which could, through dilution, nullify the effectiveness of neutralizing antibodies present in the same pools and reduce the impact of subsequent viral removal and inactivation processes during industrial fractionation. This can contaminate thousands of vials produced from this raw material, which will ultimately be administered to multiple recipients[14-15].

In contrast, the risk of B19V transmission through labile blood components, which are not routinely tested for non-enveloped viruses, is generally lower, as viremic units are transfused to single recipients. Thus, as also recently underlined by ECDC, introducing NAT testing of labile blood components for B19V is not currently justified. Although B19V DNA can persist for more than six months post-seroconversion, the titer levels are usually low, likely representing naked DNA rather than active virions. Most donors with ongoing B19V infection have protective antibodies, further reducing transmission risk, and approximately 70% of individuals are already immune to B19V, decreasing the likelihood of transfusion-transmitted infection[16,24].

Countries have adopted different strategies to manage B19V transmission risks. In the Netherlands, blood components from donors can be deemed "safe for B19V" if the donor tests positive for IgG antibodies in two samples taken in a six-month interval, allowing donor eligibility after a six-month deferral[16,22]. Conversely, the UK adopts a more flexible approach, allowing readmission of B19V-positive donors if more than four weeks have passed since symptom resolution and, where performed, B19V DNA testing is positive[25]. Both strategies aim to

protect recipients but differ in their methods, with the Netherlands emphasizing longer deferral periods and the UK allowing earlier donor readmission.

COMPREHENSIVE MEASURES AND EPIDEMIOLOGICAL ANALYSIS OF PARVOVIRUS B19 IN THE ITALIAN TRANSFUSION SYSTEM

In Italy, the safety of blood supply is ensured through a framework of prevention and control measures. These measures include a system based on voluntary, non-remunerated blood donors, who are selected from low-risk categories and individually assessed for risk factors related to communicable diseases based on eligibility criteria established by current evidence. Each donation undergoes comprehensive biological qualification tests to ensure the safety of the blood components, according to directive 2004/33/EC, and Italian regulations. In the context of plasma for fractionation, donations are pooled into production batches, with volumes defined in the Plasma Master Files (PMFs) of fractionation companies, and further tested and manufactured in compliance with the European Pharmacopoeia, including B19V screening in mini-pool, as described above.

Given the findings reported in the ECDC questionnaire, the Italian National Blood Center initiated a survey involving fractionation companies holding national plasma manufacturing agreements with the Regions and Autonomous Provinces (RAPs). To assess the epidemiological trend of B19V, an analysis was performed using regional data from 2018 to 2024, recorded by fractionation companies. This analysis included source and recovered plasma units collected by blood establishments and blood collection units in RAPs intended for industrial processing to manufacture PDMPs and S/D plasma.

The B19V positivity rate in plasma units was calculated by the ratio between a) the number of positive units detected and b) the total number of donations made each month from January 2018 to June 2024. Positivity results refer to units with viral titers above the acceptability thresholds defined by the companies, which were subsequently excluded from processing. It is important to note that the epidemiological data presented here may underestimate the true prevalence of B19V in the Italian donor population, as it only reflects units detected as positive during the testing phase, excluding those with viral titers below the defined thresholds.

Preliminary data analysis revealed two peaks in B19V positivity: one in 2018, with 46 positive units, and another in 2019, with 86 positive units. The highest peak occurred in March 2024, with 138 positive units. The ratio of B19V-positive units to total donations (subsequently sent to fractionation companies) in a given month showed peaks of 19.8 and 39.4 positive units *per* 100,000 donations in May 2018 and June 2019, respectively. Additional peaks were recorded in June 2020, with 14 positive units *per* 100,000 donations, and more recently in June 2023 and March 2024, with 10.3 and 59.4 positive units *per* 100,000 donations, respectively (**Figure 1**).

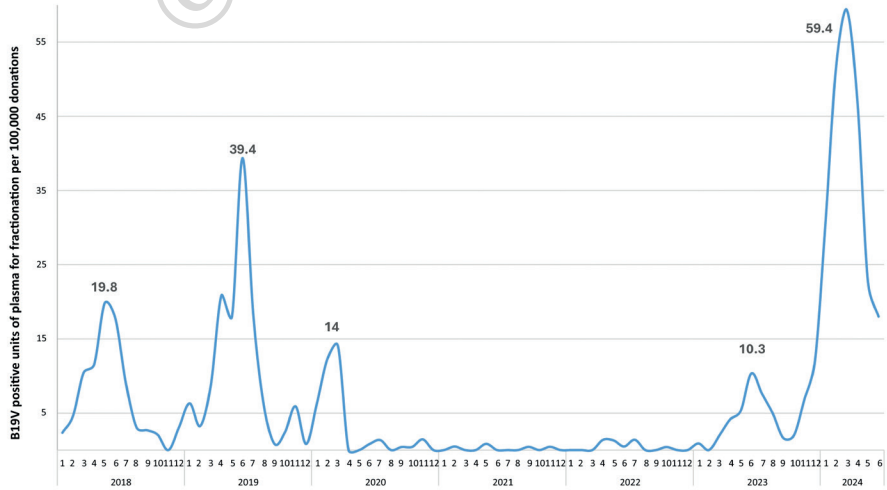

Figure 1 - B19V positive units of plasma for fractionation *per* 100,000 donations occurred in Italy in the period 2018-2024

The macro-geographic distribution of B19V-positive units (**Figure 2**) showed a fluctuating trend between 2018 and 2020, with significant peaks in Sicily and Sardinia, where 59.7 and 62.1 positive units *per* 100,000 donations were recorded in 2018 and 2019, respectively. From 2020 to 2022, these values declined, reaching near zero, increasing again in 2023. In the first half of 2024, during the testing phase, fractionation companies detected 134.5 positive units *per* 100,000 donations in the North-East, 80.4 positive units *per* 100,000 donations in the North-West, 59.3 positive units *per* 100,000 donations in the Central regions, and 46.5 positive units *per* 100,000 donations in the South of the Country. In Sicily and Sardinia, the number was 8.3 positive units *per* 100,000 donations (**Figure 3**).

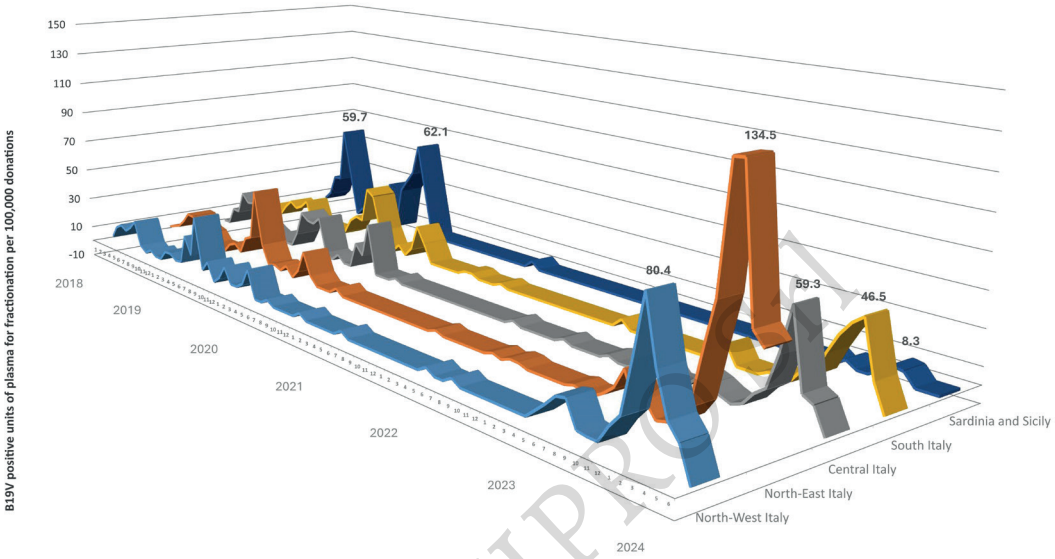

Figure 2 - B19V positive units of plasma for fractionation *per* 100,000 units donated in the related month in Italy, *per* geographical area, between 2018-2024

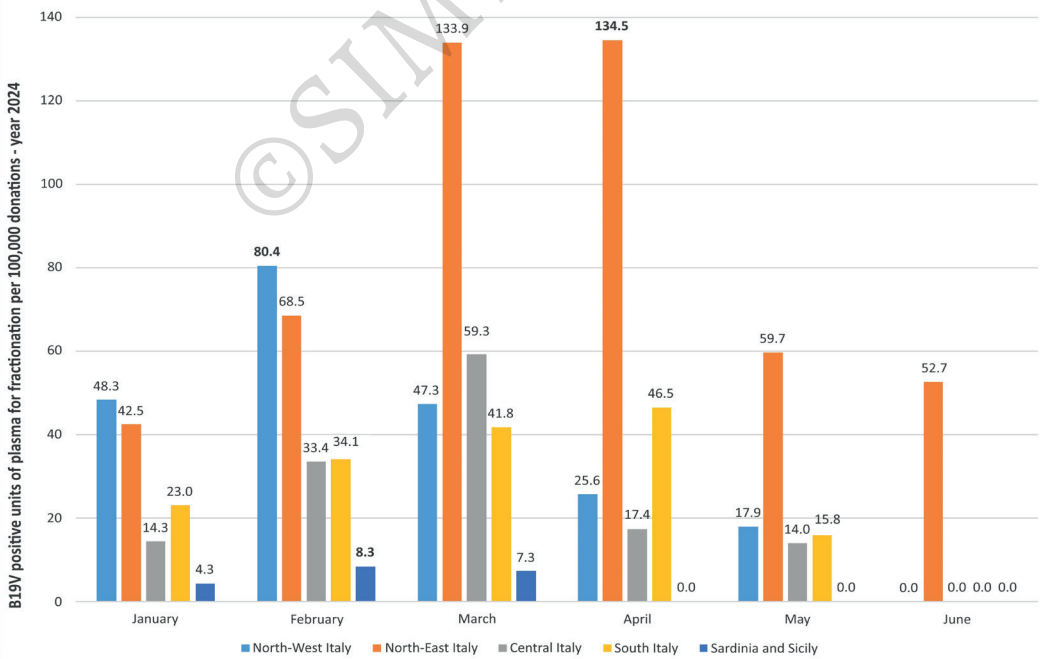

Figure 3 - B19V positive units of plasma for fractionation *per* 100,000 units donated in Italy, *per* month and *per* geographical area, January to June 2024

Specifically, in March and April 2024, the Northeast region recorded two peaks of 133.9 and 134.5 positive units per 100,000 donations, respectively. Regional data for the first six months of 2024 revealed B19V-positive units in most of the Italian RAPs: 12 out of 21 in January (with a minimum of 5.5 positive units *per* 100,000 donations in Sicily and a maximum of 150.7 in the Autonomous Province of Bolzano), 18 out of 21 in February (with a minimum of 5.5 in Sicily and a maximum of 171.1 in Piedmont), 16 out of 21 in March (with a minimum of 9.7 in Sicily and a maximum of 363.3 in Umbria), 9 out of 21 in April (with a minimum of 25.8 in Marche and a maximum of 357.4 in the Autonomous Province of Bolzano), 7 out of 21 in May (with a minimum of 51.9 in the Autonomous Province of Bolzano and a maximum of 168.9 in Liguria). In June (preliminary data), B19V-positive units were detected only in Emilia-Romagna, with 245.5 positive units *per* 100,000 donations.

PROPOSED MANAGEMENT OF DONORS FOUND TO BE POSITIVE BY B19V DNA IN ITALY

The actions to be taken in case of donations found positive for B19V during the manufacturing process are summarized in the accompanying algorithm (**Figure 4**). In the absence of specific studies assessing the duration of infectivity of blood components following donor infection, we recommend, as a precautionary measure, to apply a deferral period of 6 months from the date of donation or in case of infection from donor history in absence of tests, for donors who turned out to be viremic upon investigations conducted by the fractionation company or for donors reporting confirmed acute infection, respectively.

Additionally, in situations where a donor has a history of contact with individuals infected with B19V, a deferral should be considered based on a thorough medical assessment. This assessment should take into account the

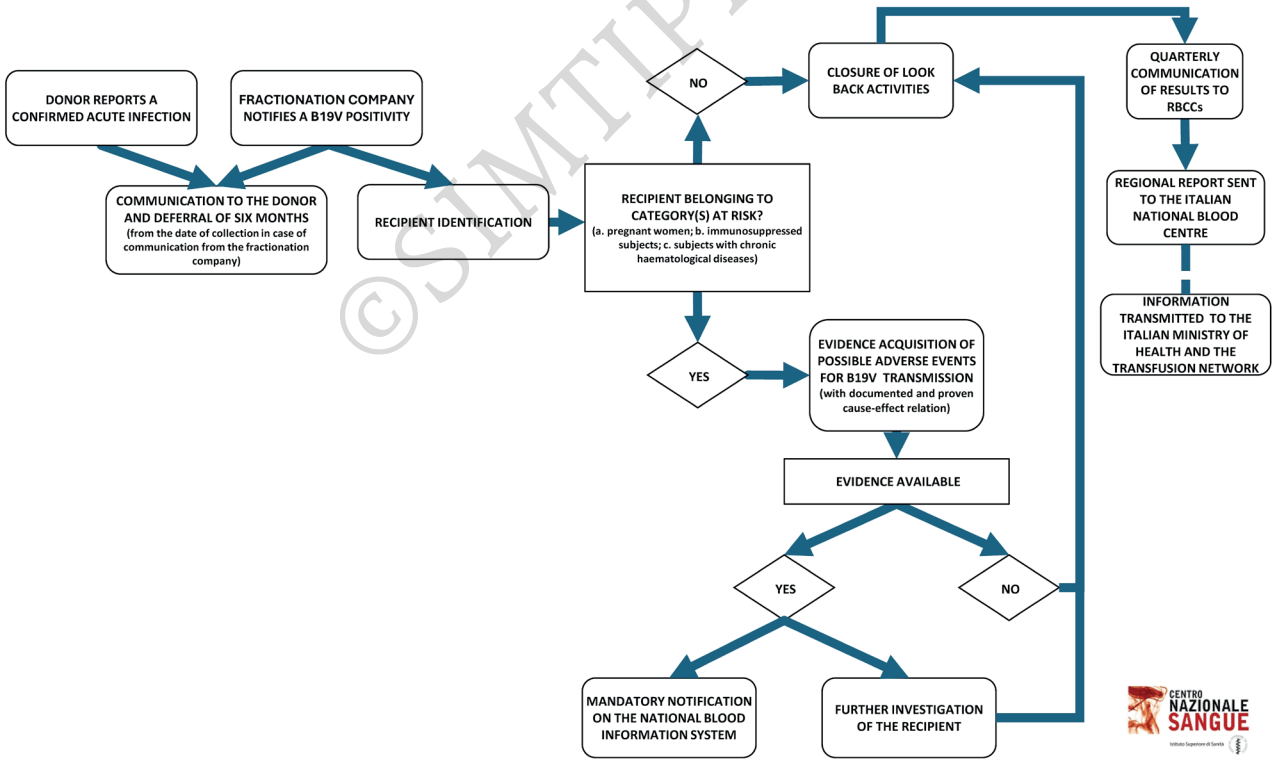

Figure 4 - Algorithm for look back activities following a reported B19V positivity

donor's previous history of B19V infection and whether the contact occurred during the post-infectious phase (i.e., after the appearance of the rash).

Moreover, we recommend tracing the recipient(s) of labile blood components derived from the same donation unit from which the plasma, subsequently sent for fractionation, was obtained. If the recipient is at moderate risk according to ECDC guideline, it is suggested to collect evidence of any adverse events potentially related to B19V transmission, in collaboration with the physician responsible for the clinical management of the patient. In all other cases, no further investigation is currently justified, considering the generally benign course of the infection and the fact that 70 percent of the adult population already has natural protective immunity against the virus.

RESEARCH PRIORITIES

Understanding transfusion-transmitted B19V is crucial for improving transfusion safety and patient outcomes. In our opinion research should focus on specific points, as discussed below.

1. Studies needed to understand the impact of transfusion-transmitted B19V, especially among vulnerable patients. More information of this issue will derive from the look back of the patients who have received labile blood components from viremic donors, as assessed by B19V DNA screening conducted on plasma for fractionation.
2. Further research is essential to determine the duration of B19V infectivity in blood donors after infection. Accurately understanding how long B19V remains infectious in blood products is crucial for establishing evidence-based deferral periods that ensure safety while minimizing unnecessary reductions in the donor pool.
3. Regarding preventive strategies, the feasibility and cost-effectiveness of implementing universal B19V screening for blood donations in geographic areas with high transmission rates, as well as the targeted screening of donations intended for vulnerable patients, need to be explored.
4. There is a need to standardize the risk assessment tools, in order to harmonize practices across different regional health authorities and other institutions to ensure consistent and effective prevention of B19V transmission in the transfusion setting.

CONCLUSIONS

The significant increase in B19V-positive plasma units observed in Italy during early 2024 underscores the cyclical nature of B19V epidemiology and the associated risks of transfusion-transmitted infections. While routine NAT testing for B19V in individual blood donations may not be justified at this time, advanced screening protocols for plasma intended for industrial fractionation are essential to ensure the safety of PDMPs. However, the widespread implementation of B19V screening for fractionation introduces the complex challenge of managing donors at risk for transmission. Addressing this issue requires both the deferral of these donors and the implementation of targeted look-back procedures to trace and monitor recipients of labile blood components derived from these donations. This must be carefully balanced to ensure recipient safety while retaining donors, and it highlights the operational and ethical challenges that transfusion services must address, creating new opportunities for research.

The data collected from Italian blood establishments and fractionation companies reveal notable regional disparities in B19V positivity rates, emphasizing the need for tailored strategies to address these differences effectively. The proposed deferral periods for B19V-positive donors, combined with the recommended monitoring of recipients at moderate risk, provide a comprehensive framework for maintaining transfusion safety.

As B19V infections continue to pose challenges across Europe, the insights gained from Italy's experience offer valuable guidance for other countries facing similar epidemiological trends. Moving forward, the adoption of evidence-based practices, along with ongoing monitoring and targeted research initiatives, will be essential for safeguarding Public Health and maintaining high standards of blood safety.

AUTHORS' CONTRIBUTIONS

All the Authors contributed to the writing of the manuscript, revised the work and approved the final version.

The Authors declare no conflicts of interest.

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