# Human Parvovirus B19 and blood product safety: a tale of twenty years of improvements

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## Introduction

The establishment of systems to ensure a safe and sufficient supply of blood and blood products for all patients requiring transfusion is a core issue of every blood programme.

A spectrum of blood infectious agents is transmitted through transfusion of infected blood donated by apparently healthy and asymptomatic blood donors. Recent emerging-infectious-disease threats include West Nile virus[1,2], chikungunya[3], babesia[4], dengue[5], hepatitis E virus[6], and variant of Creutzfeldt-Jakob disease[7].

Parvovirus B19 (B19V), long known to be the causative agent of *erythema infectiosum* (fifth disease), is not a newly emerging agent. However, it deserves discussion because it may be present in blood and in plasma products, can circulate at extraordinarily high titres, can infect recipients, and, in some cases, can cause severe disease[8]. Its potentially severe pathological effects have become more apparent in the past decade with the widespread use of (pooled) plasma-derived medicinal products and are the main reason for the uneasy relationship between transfusion medicine specialists and B19V[9].

The aim of this review is to analyse the role played by this virus in compromising safety in transfusion medicine and the progressive measures to reduce the risks associated with the virus.

## The virus

B19V, a member of the *Parvoviridae* family, *Parvovirinae* subfamily, Erythroparvovirus genus, and Primate erythroparvovirus 1 species[10], is a small non-enveloped DNA virus, discovered in 1975 by Yvonne Cossart in the blood of a healthy blood donor[11]. The name parvovirus originates from the Latin word *parvum*, which means small. In fact, parvoviruses are among the smallest known viruses with a virion diameter of 18-26 nm[12]. Their structure is simple: the icosahedral virion consists only of proteins and linear single-stranded-DNA genome (length 5-6 kb) with hairpin structures at both ends. The hairpins are palindromic and the 3′-end can fold and function as a primer during viral replication[12] (Figure 1). Three B19V genotypes have been identified based on isolates having nucleotide divergence greater than 10%. Genotype 1 is the most prevalent type currently circulating worldwide and is the B19V prototype[13]. It largely replaced genotype 2 viruses, which were common in Europe half a century ago[14] and, therefore, has been sporadically found in plasma donations from Europe and North America[15]. Genotype 3 has been found predominantly in West Africa (Ghana) thus implying a different and longer evolutionary history, probably rooted in Africa[16]. Interestingly, recent studies found B19V genotype 3 in samples from Europe, Asia, and Brazil thus raising the question that this genotype may be more widely distributed outside Africa than previously believed[17].

In its infection cycle, the virus links to a specific receptor on the surface of host cells[18] (i.e. P blood group antigen globoside-4 [Gb4] in the case of human B19V) and is transported into the cell by endocytosis. Inside the host cell, the virion is transported to the nucleus where its

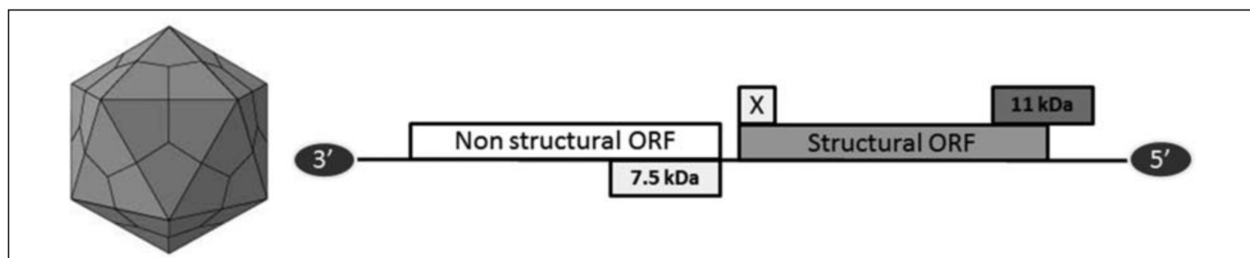


**Figure 1** - Schematic representation of Parvovirus B19 icosahedral virion and genome structure. ORF: open reading Frame; kDa: kiloDaltons; X: Region X.

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replication takes place. Parvoviruses do not encode their own DNA-polymerase and are, therefore, all dependent on host cell polymerase and the S-phase of dividing cells[12]. Gb4 is also responsible for the phenomenon of B19V haemagglutination[19]. Individuals who lack the blood group P antigen on their erythroid cells are not, therefore, susceptible to B19V infection[20]. The B19V receptor is also present on platelets, cardiac, synovial, renal and thyroid cells as well as on hepatic progenitors[8]. Interestingly, between 1989 and 1992, B19V replication was shown in a primary culture of erythroid cells derived from foetal liver[21], in haematopoietic progenitor cells from a blood donor[22], and in human umbilical cord blood cells[23].

The bone marrow cell tropism of B19V is now well recognised as an erythroid progenitor cell tropism[24]. In fact, erythroid progenitor cells are susceptible to infection and this susceptibility increases with differentiation. Therefore, the destruction of the source of mature red blood cells will result in dramatically low haematocrit levels and a temporary state of anaemia. Naturally, in patients with disorders that shorten red blood cell half-life the clinical picture can be more severe and there may also be a transient aplastic crisis[20]. On the other hand, the concentration of Gb4 on non-haematopoietic tissues does not appear sufficient for active infection, and other factors may be required for virus internalisation and replication[19].

**Markers of Parvovirus B19 infection**

B19V viraemia occurs 1 week after exposure and usually lasts about 5 days, with virus titres peaking in the first 2 days (Figure 2). IgM antibodies against B19V are detected late in the viraemic stage. They appear 10 to 14 days after the infection and can persist for up to 5 months but, in some patients, they can last even longer (Figure 2)[25]. Specific IgG antibodies are detectable about 15 days after infection, remain high for several months and persist long-term[25].

Parvovirus serology (anti-B19V immunoglobulin [Ig] M and IgG antibodies) can be determined using enzyme-linked immunosorbent assay (ELISA), radioimmunoassay, chemiluminescence or immunofluorescence[25].

IgA antibodies are also detectable for a short period and may play a protective role in the respiratory tract[26]. In addition, long-term B19V-specific IgE antibodies have been found, but their biological functions are unclear[27].

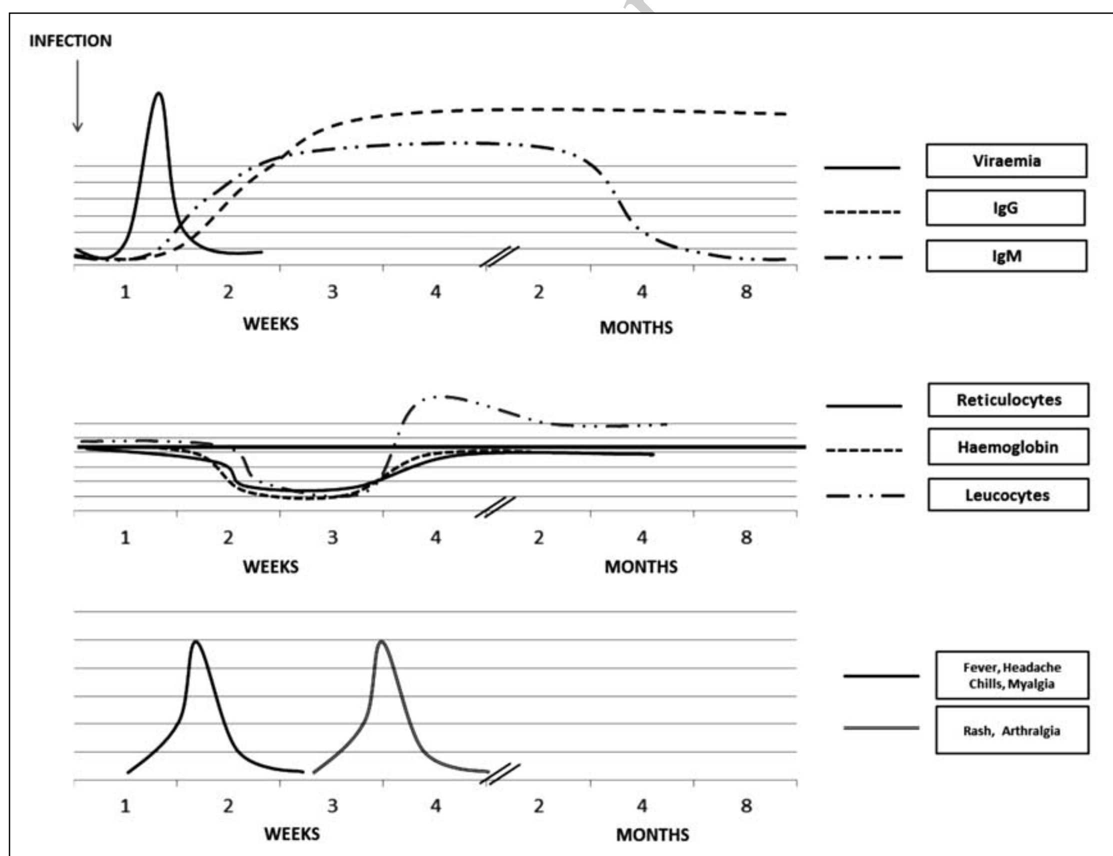


**Figure 2** - Clinical, haematological and viral course after B19V infection in immunocompetent subjects. B19V: Parvovirus B19; IgG: immunoglobulin G; IgM: immunoglobulin M.



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Given that there are different epidemiological patterns of infection, the seroprevalence of B19V-IgG varies widely from approximately 2% to 21% in children from 1 to 5 years of age, from 30% to 40% in adolescents, from 40% to 60% in adults, and more than 85% in elderly populations[28,29]. Neutralising IgG antibodies generally appear about 2 weeks after infection and persist lifelong (Figure 2). The development of the antibody response corresponds to virus clearance and also, in the vast majority of immunocompetent individuals, protection from disease[25]. The protective role of antibodies against B19V is demonstrated by the efficacy of commercial immunoglobulins used to treat B19V infection in immunocompromised subjects, in whom, due to the absence of antibodies, viral levels can be even higher than $10^{12}$ copies/mL[30].

Results of IgM testing are particularly difficult to interpret[31]. Standardisation among laboratories is lacking. Even in a single laboratory, sensitivity and specificity partly depend on the operator's skills. High-level viraemia in acutely infected subjects may cause virus-antibody complexes, which will result in a false-negative IgM test result[31].

In this setting, polymerase chain reaction (PCR) analysis or alternative nucleic acid amplification technology (NAT) assay (e.g., transcription-mediated amplification) may be a better diagnostic tool as viral titres can reach more than $10^{12}$ genome equivalents (geq) per mL during acute B19V infection. On the other hand, in chronic B19V infection, viral DNA can persist in the host without the presence of B19V IgM or IgG. In the immunocompetent host, viral DNA is detectable for at least 1 month after infection[30], but can persist even longer at low levels[32]. Therefore, B19V DNA, detected by qualitative PCR analysis, is not always indicative of recent infection[25]. This assay has been set up to include an internal control to eliminate false negative results caused by the presence of inhibitors in plasma. It is very useful for plasma pools for fractionation and could also be exploited for clinical diagnosis in primary red cell aplasia, in which the antibody response is depressed, or for the detection of recently discovered variant erythroviruses in human disease[33].

Finally, as far as the identification of B19V genotypes 2 and 3 is concerned, many commercial and in-house PCR methods have shown lower sensitivity or failure to detect either or both of these genotypes[12,15].

## Clinical features and therapy

Many individuals with B19V infection are asymptomatic (about 25% of adults and 50% of children during an outbreak), or manifest mild, non-specific, cold-like symptoms that are never linked to the virus[34,35] (Figure 2). Most patients with B19V infection do not require laboratory tests or therapy because symptoms are mild and the illness resolves over 5-7 days.

The pattern of clinical disease is strongly influenced by the haematological and immunological status of the host[36]. B19V infection may have a serious clinical outcome in three categories of susceptible recipients: (i) patients with shortened red cell survival (e.g. those with thalassaemia major, sickle cell disease, or other haemolytic diseases); (ii) immunocompromised patients (whether previously exposed to B19V or not); and (iii) pregnant women.

The most common clinical manifestation in children, especially those aged 4-11 years, is erythema infectiosum causing a "slapped-cheek" facial rash, extending later to the trunk and limbs ("gloves and socks" syndrome) and accompanied, in a few cases, by fever, headache, coryza, nausea, and diarrhoea. Joint involvement, transient haemolytic anaemia, and encephalitis may also occur.

In adults, polyarthropathy characterised by sudden symmetrical arthralgia is more common than rash. Some patients may present acute anaemia but those with underlying haemolytic disorders may develop a transient aplastic crisis[37]. Persistent B19V infection can also cause severe anaemia. The first patient recognised to have this condition had an underlying combined B- and T-cell immunodeficiency with low immunoglobulin levels (Nezelof syndrome)[38]. Patients with human immunodeficiency virus (HIV) infection can also develop pure red blood cell aplasia due to B19V infection; in these patients, B19V titres can be as high as $10^{12}$ copies/mL.

During pregnancy (weeks 9 to 20) B19V infection is associated with an approximately 10% incidence of foetal loss, which is rare after the 20th week. B19V infection of foetal cardiac tissue may lead to myocarditis and heart failure, which can result in a hydrops foetalis, neurological foetal abnormalities, or congenital anaemia[34].

Other complications associated with B19V infection include encephalopathy, epilepsy, meningitis, myocarditis, dilated cardiomyopathy, and autoimmune hepatitis[34].

There are no specific antiviral drugs or vaccines against human parvovirus infection. The (aplastic) anaemia can be treated through intravenous immunoglobulin therapy, possibly repeated in chronic and persistent infections[25], or red blood cell transfusion, when necessary[39]. On the other hand, in immunocompetent patients, treatment is unnecessary and infections are self-clearing.

## Virus transmission and blood product safety

Human B19V circulates worldwide, is mildly contagious, and may occur sporadically or in epidemics. In temperate climates, epidemic manifestations occur more commonly in late winter, spring or early summer[36].



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B19V is mainly transmitted through the respiratory route and its DNA is contained in respiratory secretions at the time of viraemia, when infectivity is highest[40]. Transmission in the household, at day care, and in school is common, although the virus can also be transmitted vertically[41] and via blood transfusion and organ/bone marrow transplantation[14].

The risk of vertical transmission varies between 33% and 51% following acute infection of pregnant women with reported adverse foetal outcomes in 3% to 12% of cases[41]. There is evidence that transmission of severe B19V infection may occur at the time of solid organ or haematopoietic transplantation and cause serious complications such as aplastic crises, pneumonia, and multiorgan failure[42-45]. Iatrogenic transmission of B19V through blood products is possible because high-level viraemia regularly occurs during primary infection and more than $10^{12}$ geq/mL are often found in the blood of asymptomatic individuals during the early phase of acute infection[46].

***Plasma-derived medicinal products***

Although there have been very few reports of clinically relevant B19V infection resulting from the transfusion of a blood component containing the virus[47], B19V is a frequent contaminant of blood and plasma donations and has been transmitted by plasma-derived medicinal products[13,48,49]. Interestingly, both a temporal correlation between infusion of plasma-derived medicinal products and viraemia, seroconversion and/or non-clinically significant infections and blood product viral contamination have been documented. Other studies showed that the prevalence of B19V-specific antibodies was much higher in groups receiving clotting factors than in control groups[50].

Transmission via plasma-derived medicinal products can occur because of incomplete physiological clearance of virus, high-level viraemia in acutely infected individuals[14], and the resistance of B19V to most viral inactivation procedures used in the manufacturing of blood-derived products[51-55].

B19V is a good model for new emergent viruses capable of infecting blood products because of their properties of physical resistance. In fact, B19V DNA is detectable in 50% to 80% of non-virally inactivated factor VIII concentrates and in 30% to 50% of solvent/detergent-inactivated factor IX concentrates, respectively[12,56]. B19V was also detected in two of three unheated batches of clotting factor preparations and in 20% to 25% of solvent/detergent-treated batches, while the fractionation process used to obtain albumin preparations is apparently more efficient at eliminating the virus[57-62]. B19V DNA sequences were also detected in 16%[63] to 28%[64] of factor VIII concentrates. A more recent German study detected B19V DNA in 26% of different coagulation factor concentrates produced between 2007 and 2008[65]. Soucie and colleagues found that compared to populations not exposed to blood or blood products, the studied population receiving plasma-derived medicinal products was 1.7 times more likely to have IgG antibodies to B19V[66]. Another study demonstrated that, overall, 25% of albumin samples, 100% of factor VIII concentrates, and 20% of intravenous and 75% of intramuscular immunoglobulin preparations contained B19V DNA[67]. The viral load in those samples ranged from $1\times10^2$ to $1\times10^6$ geq/mL. Alter *et al.* reported a high prevalence (over 60%) of B19V DNA in factor IX, factor VIII, and prothrombin complex concentrates, as well as plasma pools with viral loads of $1\times10^2$ to $1\times10^8$ geq/mL[9].

In conclusion, the multiple reports of B19V transmission by pooled-plasma products were almost always documented by recipient seroconversion in asymptomatic cases and less frequently by clinical diagnosis of B19V-related disease associated with positive B19V test results[68]. It is also worth noting that according to a recent interim report from a prospective clinical study on the incidence of factor VIII inhibitors in previously untreated patients during prophylaxis, on-demand treatment, and surgical procedures with a plasma-derived, human, von Willebrand factor-stabilised FVIII product, the 57 adverse events rated "serious" probably or possibly related to factor VIII treatment reported in 24 subjects also included 14 cases of asymptomatic parvovirus B19 seroconversion[69].

***Inactivation/removal steps and B19V***

In order to obtain safe plasma-derived medicinal products, the processes to produce these products include steps to inactivate or remove viruses. B19V has been shown to be susceptible to chromatography, pasteurisation (10 hours at 60 °C), steam (vapour heat), exposure to pH 4 (occasionally used during the manufacture of immunoglobulins), and (only partially)[55] to small-pore-size nanofiltration[70]. It was shown that porcine parvovirus (a model for human parvovirus B19) could be effectively inactivated with riboflavin/UV light suggesting that B19V could also be inactivated. On the other hand, amotosalen/UVA light is not effective on porcine parvovirus; its limited effectiveness against certain of these viruses was demonstrated when transmission of hepatitis E virus occurred via transfusion of a plasma product. Levels of viral load of non-lipid-enveloped viruses, such as B19V, could be reduced by 4 log or more through methylene blue treatment, while other viruses, such as hepatitis A virus, are not affected[70]. Transfusion transmission of B19V was also reported with solvent/detergent-treated plasma[54].

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**Relationship between viral load in plasma and probability of transmission by blood products**

To date, no B19V transmission from pooled-plasma products has been documented when less than $10^3$ to $10^4$ IU/mL B19V DNA are present in an infused product[13]. There is still some doubt as to the reason for this lack of infectivity. It may be due to an inadequate amount of infused infectious virions, a neutralising effect from B19V antibodies present in other plasma units contained in the plasma pool, or a combination of these factors. Recipient factors may also play a role because it has been reported that B19V antibodies protect against B19V re-infection, and most of the adult population is B19V-seropositive as a result of previous infection[71].

To reduce the potential risk of transmission, the US Food and Drug Administration proposed a limit of $10^4$ geq/mL for the production of plasma pools destined for all plasma derivatives[72]. Similarly, the European Pharmacopoeia has imposed a limit of $10^4$ UI/mL of B19V in anti-D immunoglobulins and pooled virus-inactivated plasma[73].

***Labile blood products***

As far as cellular blood products are concerned, B19V DNA levels lower than $10^4$ IU/mL might not be clinically significant while the transfusion of labile blood products with B19V titres greater than $10^7$ IU/mL has been associated with transmission of B19V infection[13,25]. However, susceptibility to infection could be highly dependent on the presence or absence of neutralising antibodies in the recipient[74]. Symptomatic infections have been reported in a few case series and linked donor-recipient studies have confirmed that in most cases B19V transfusion-transmitted infections are clinically irrelevant[68] while vulnerability to serious B19V-related haematological disorders is dependent on the patients' underlying diseases[50].

Studies in different countries found B19V DNA in 1% of all blood cell preparations and blood products transfused to patients in a haematology ward[49], in 0.9% of standard blood components[49] (in 2% of pooled-plasma products and in 0.7% of single-donor products)[49], in 0.006% of blood donations[75], in 0.14% of single-donor blood products[76], and in 0.16% of plasma samples[77]. The prevalence of B19V DNA in plasma pools ranged from 0.024%[78] to 97%[79]. Interestingly, another study demonstrated that, overall, 85% (60% to 100% depending on the manufacturer) of plasma pools contained B19V DNA[67]. The percentage of the plasma pools positive for B19V DNA that were also positive for IgG was 100%[79,80], while the percentage of the same plasma pools also positive for IgM ranged from 23%[80] to 65%[79].

There are several reasons for the very different figures in diverse studies: first, the epidemiological settings are different; second, the rates of detectable viral DNA are related to the sensitivity of the methods used; and third, there are seasonal variations in transmission and, therefore, in viraemia[49].

***Strategies for screening donors or plasma pools***

Since April 2000, all blood donations started to be screened by a B19V minipool real-time NAT in German Red Cross Centres and four areas in Austria[51]. Since 2004, Polish blood donors have also been tested for B19V DNA. Screening has been performed in donors of plasma for fractionation for anti-D and anti-HBs production, and donors of erythrocytes used for immunisation[81]. In 2008, to reduce the risk of B19V transmission through contaminated blood for transfusion and plasma-derived medicinal products, Japanese Red Cross Blood Centres introduced B19V antigen screening by chemiluminescent enzyme immunoassay for all donated blood. This test has a sensitivity of approximately $10^7$ IU/mL. Positive samples are then excluded from the 20-pool-screening triple NAT to reduce the risk of cross-contamination during NAT[82]. There has been a subsequent expansion of B19V DNA screening of pools of plasma used to manufacture plasma derivatives in many countries[83]. The combined strategy of high-titre-B19V PCR screening and viral decontamination during the plasma manufacturing process has significantly increased the margin of B19V safety of plasma-derived medicinal products[84].

***Prevalence of B19V in blood donors and patients***

Blood donor screening for B19V is feasible using B19V antigen assays or NAT. Many commercial or in-house real-time NAT systems are available[83]. The risk of exposure to a high-load B19V viraemia during a window period is relatively small, but during epidemic periods, the incidence of parvovirus in the blood can be as high as one in 260 donors[85].

Several studies published between 1995 and 2014[16,51,68,78-82,84,86-117] show that the prevalence of B19V in blood donor populations ranges from 6%[92] to 79.1%[112] for IgG (92% in donors older than 61 years[101]), from 0.72%[98] to 7.53%[96] for IgM, from 0.01%[82] to 15.3%[87] for IgM+IgG, and from 0%[89] to 1.3%[16,89,102] for B19V DNA (see Table Ia and Table Ib).

Table II lists several studies, published between 1988 and 2013, on the prevalence of B19V in patients with congenital bleeding disorders[66,118-127]. The prevalence of B19V in these patients ranged from 31%[126] to 97%[123]. Interestingly, the range for each specific disorder was from 21%[120] to 93%[123] for haemophilia A, from 35.5%[66] to 97%[123] for haemophilia B, and from 37.9%[66] to 80%[123] for von Willebrand disease.



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**Table Ia** - Prevalence of parvovirus B19 reported in blood donors, blood donations and plasma pools.

| Continent | Country | Prevalence | | | | Subject of study | Study author and year of publication |
|---|---|---|---|---|---|---|---|
| | | *IgG (%)* | *IgM (%)* | *IgM+IgG (%)* | *B19V DNA (%)* | | |
| AFRICA | Ghana | - | - | - | 0.8 | Blood donors | Compston LI, 2008[86] |
| AMERICA | Canada | 24.3 | 4.7 | 15.3 | - | Blood donors | Wasfi S, 1996[87] |
| | Brazil | 60 | - | - | 1 | Blood donors | Slavov SN, 2012[88] |
| | Brazil | 57.4 | - | - | 0 | Blood donors | Slavov SN, 2012[89] |
| | Chile | - | - | - | 0.84 | Blood donors | Lévican J, 2011[90] |
| | Chile | 54.8 | - | - | - | Blood donors | Gaggero A, 2007[91] |
| | USA | 6 | - | - | - | Blood donors | O'Bryan TA, 2010[92] |
| | | - | - | - | 0.84 | Blood donors | Kleinman SH, 2009[68] |
| | | - | 1 | - | - | Blood donors | Doyle S, 2000[93] |
| | | - | - | - | 0.88 (100: IgG+ 23: IgM+) | Blood donations | Kleinman SH, 2007[80] |
| ASIA | China | - | - | | a) 54 (100: IgG+ 65.6: IgM+) b) 97 c) 21.6 | a) Plasma pools; b) apheresis donors; c) plasma derivatives | Zhang W, 2012[79] |
| | | 24.6 | 6.9 | 2.5 | 0.58 | Blood donors | Ke L, 2011[94] |
| | | 55.43 | - | - | - | Blood donors | Wei Q, 2006[95] |
| | India | 27.96 | 7.53 | 2.40 | - | Blood donors | Kumar S, 2013[96] |
| | | 39.9 | - | - | - | Blood donors | Kishore J, 2010[97] |
| | Japan | - | - | 0.01 | 0.04 | Blood donors | Sakata H, 2013[82] |
| | | 67.9 | 0.72 | - | 0.2 | Blood donors | Ihara T, 2013[98] |
| | | - | - | - | 0.01 | Blood donors | Matsukura H, 2008[99] |
| | | - | - | 0.11 | - | Blood donors | Wakamatsu C, 1999[100] |
| | | 40 (donors 16-30 years old) 92 (donors >61 years old) | - | - | 0.250 | Blood donors | Tsujimura M, 1995[101] |
| | Saudi Arabia | 39.3 | 0 | 44.6 | 0 | Blood donors | Obeid OE, 2011[102] |
| | South Korea | - | - | 60.1 | 0.1 | Blood donors | Oh DJ, 2010[103] |
| | Thailand | 20.16 | - | - | - | Blood donors, children | Poovorawan Y, 2000[104] |

B19V: parvovirus B19; IgG+: immunoglobin G positivity; IgM+: immunoglobin M positivity; IgG&IgM+: immunoglobin G and immunoglobin M positivity; geq/mL: genome equivalents/mL.

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## Other parvoviruses, blood donors, and blood products

B19V was considered to be the only human pathogenic parvovirus until the recent discovery of *Bocaparvoviruses* and Parvovirus 4 (PARV4), whose epidemiology and disease association are still poorly understood[128].

PARV4 is a member of the *Primate Tetraparvovirus 1* species[10], *Parvoviridae* family, discovered in 2005 in plasma from an intravenous drug user, with symptoms consistent with acute HIV infection but who was confirmed to be HIV-RNA negative[129]. A related virus variant Parvovirus 5 (PARV5) was identified in plasma pools used in the manufacturing process of plasma-derived medicinal products[130]. Later, the name PARV5 was changed to PARV4 genotype 2. In 2008, a third genotype of PARV4 was found in two patients in sub-Saharan Africa with acquired immune deficiency syndrome[131]; non-parenteral transmission might contribute to its transmission in this area[132]. The virus was detected in plasma pools used in the manufacturing process of plasma-derived medicinal products, particularly those from the United States[129,133] and Asia[134], and also in clotting factor concentrates, namely preparations of factor VIII and IX[134,135]. In contrast, three studies carried out in France and Germany did not detect any PARV4 DNA positive samples in a large number of plasma donations, minipools or coagulation factor concentrates[65,136]; it is still unclear whether these negative results have seasonal or geographical explanations[12].

Interestingly, products manufactured in the early 1970s were found to be positive for PARV4, and in



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**Table Ib** - Prevalence of parvovirus B19 reported in blood donors, blood donations and plasma pools.

| Continent | Country | Prevalence | | | | Subject of study | Study author and year of publication |
|---|---|---|---|---|---|---|---|
| | | *IgG (%)* | *IgM (%)* | *IgM+IgG (%)* | *B19V DNA (%)* | | |
| EUROPE | Belgium | - | - | - | 0.16 | Blood donors | Thomas I, 2003[105] |
| | France | - | - | - | 0.024 | Plasma pools | Petermann R, 2010[78] |
| | Germany | 0.88 | - | - | 0.014 | Blood donations | Hitzler WE, 2002[106] |
| | | - | - | - | 0.125 | Blood donations | Weimer T, 2001[84] |
| | | - | - | - | 0.66 | Blood donors | Juhl D, 2014[107] |
| | The Netherlands | - | - | - | 0.006 (0: IgG+ 24: IgM+ 6: IgG&IgM+) | Blood donors, plasma pools | Kooistra K, 2010[108] |
| | | - | - | - | 0.005 (70: IgG&IgM+) | Blood donors | Corcoran A, 2007[109] |
| | | - | - | - | 47 | Plasma pools | Zaaijer HL, 2004[110] |
| | Italy | - | - | - | 0.89 | Mini-pools | Gessoni G, 2007[111] |
| | | 79.1 | - | - | - | Blood donors | Manaresi E, 2004[112] |
| | Poland | | | | 0.10 | Blood donors | Grabarczyck P, 2012[81] |
| | Portugal | - | - | - | 0.12 | Blood donors | Henriques I, 2005 [113] |
| | Russia | - | - | - | 1 (100: IgG+ 1 case: IgM+) | Blood donors | Filatova EV, 2010[114] |
| | Spain | 64.7 | 0 | - | - | Blood donors | Munoz S, 1998[115] |
| | | 9.78 | - | - | - | Blood donors | Mata Rebon M, 1998[116] |
| OTHER COUNTRIES | UK, Ghana, South Africa, and Malawi | - | - | - | 0.9 UK 1.3 Ghana 0.55 S. Africa 1.25 Malawi | Blood donors | Candotti D 2004[16] |
| | Germany and Austria | - | - | - | 0.0127 >$10^5$ geq/mL 0.26<$10^5$ geq/mL | Blood donors | Schmidt M, 2007[51] |
| | Belgium and Tunisia | Belgium: 74 Tunisia: 65 | - | - | - | Blood donors | Letaïef M, 1997[117] |

B19V: parvovirus B19; IgG+: immunoglobin G positivity; IgM+: immunoglobin M positivity; IgG&IgM+: immunoglobin G and immunoglobin M positivity; geq/mL: genome equivalents/mL.

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general, older concentrates were found to be more frequently contaminated with PARV4[137]. This could be due either to differences in population-based hazard of infection over time or to improvements in manufacturing processes[12].

Several studies have subsequently found PARV4 in intravenous drug users in Europe, Asia, and the United States as well as in men who have sex with men and in febrile patients[138-141]. Such cases are indicative of blood-borne transmission of PARV4. The virus has also been detected in blood donors in the United States, South East Asia, and Europe[65,130,133-135,138,141-150] (Table III). The prevalence of PARV4 in blood donor populations is not clear; studies carried out in France found that the prevalence of PARV4-DNA ranged from 0%[145] to 24%[148] in donors and from 4%[134] to 26.15%[133] in plasma-derived medicinal products. The high frequency of detection of PARV4 DNA reported in some articles[65,133,148,149] may be the result of seasonal and/or geographical epidemiological variation or lack of standardisation of detection methods.

Very little information is available regarding the clinical significance of infection with PARV4; so far, in a study of acutely infected persons with haemophilia the only repeatedly observed clinical presentation was a rash in three subjects and unexplained hepatitis in two patients[151].

Among *Bocaparvoviruses,* the *Primate Bocaparvovirus* 1 species includes *human bocavirus 1* (HBoV1)[10], which was identified in 2005 in nasopharyngeal aspirates of children with respiratory tract infections[152]. Although the routes of transmission of *Primate Bocaparvoviruses* are unknown, many parvoviruses are transmitted by inhalation or contact with infectious sputum, faeces, or urine. HBoV1 is predominantly a respiratory pathogen, whereas three



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**Table II** - Prevalence of parvovirus B19 reported in blood product recipients with bleeding disorders.

| Prevalence of B19V antibody (%) | Subjects of study | Study author and year of publication |
|---|---|---|
| a) Haemophilia A: 42.1<br>b) Haemophilia B: 35.5<br>c) vWD: 37.9<br>d) Rare factor deficiencies: 55.8 | Patients with bleeding disorders | Soucie JM, 2013[66] |
| Haemophilia: 64.2 | Patients with haemophilia, malignant disease, immunodeficiency diseases, common gynaecological ailments, pregnant women and children with malignant diseases. | Reinheimer C, 2010[118] |
| 91.8 | Patients with haemophilia | Langara H, 2005[119] |
| 21 to 48.5 | Patients with haemophilia A | Soucie JM, 2004[120] |
| a) Haemophilia A: 79.2<br>b) Haemophilia B: 83.3<br>c) Controls: 34.6<br>IgM was found in 2 patients with haemophilia.<br>No B19V DNA was found. | Patients with haemophilia | Brojer E, 1999[121] |
| 76.7 | Patients with bleeding disorders | Canales MA, 1998[122] |
| a) Haemophilia A: 93<br>b) Haemophilia B: 97<br>c) vWD: 80<br>Total 93 | Patients with bleeding disorders | Mauser-Bunschoten EP, 1998[123] |
| IgG: 84<br>IgM: 0 | Patients with bleeding disorders | Aguilar Franco C 1997[124] |
| 81.6 | Patients with haemophilia | Ragni MV, 1996[125] |
| a) Haemophiliacs: 31<br>b) Controls: 5 | Patients with haemophilic arthritis | Zakrzewska K, 2001[126] |
| a) Haemophiliacs:<br>55.5 Heated products<br>93.3 Unheated products<br>b) Healthy donors: 29.3 | Patients with haemophilia and healthy donors | Bartolomei Corsi O, 1988[127] |

B19V: parvovirus B19; vWD: von Willebrand disease.

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additional species (HBoV2, HBoV3, and HBoV4) have been found mainly in stool. A variety of signs and symptoms have been described in patients with HBoV infection, including rhinitis, pharyngitis, cough, dyspnoea, wheezing, pneumonia, acute otitis media, fever, nausea, vomiting, and diarrhoea[153]. The rate of nosocomial respiratory acquisition may be as high as 18% in hospitalised HBoV1 cases, and up to 19% of nosocomial acute respiratory tract infections are HBoV1 positive. Intrauterine infection is unlikely because of the high degree of immunity in pregnant women[153]. The seroprevalence of HBoV1 has been reported to be more than 90% in adults. However, the HBoV1-4 viral-like particles used in the ELISA have shown cross-reactivity, which might affect serological assays. Norja *et al*. found that the seroprevalence of HBoV1 was 94.9% but after removing cross-reacting antibodies the rate dropped to 68.4%[154]. Similar results were obtained by Kantola *et al*.[155], who observed that the seroprevalence of HBoV1 in adults decreased, from 96% to 59%, after removing the cross-reacting antibodies. The Kantola study found that the seroprelavence of HBoV in adults was 34% for HBoV2, 15% for HBoV3, and 2% for HBoV4[155].

Interestingly, three studies in blood donors and plasma-derived medicinal products failed to detect HBoV1 DNA[65,133,136]. This may be due to the higher frequency of HBoV1 infections among young children than in blood donors or to low-level viraemia undetectable in large plasma pools[12]. As seroprevalence studies on blood donors and blood products are limited, this issue could be an interesting and useful subject of investigation for the near future.

## Conclusions

Transfusion-transmitted human B19V is a classic example of an unresolved issue for the transfusion medicine community. The strategies used by plasma fractionators and competent authorities to ensure the safety of plasma-derived medicinal products include NAT screening of single donations and mini-pools and the adoption of multiple steps of viral inactivation and removal with solvent/detergent, super-heating (at 80 °C for 3 days), pasteurisation, and nano-filtration[156].

The current strategy of B19V-NAT plasma mini-pool screening might not be completely effective at preventing the transmission of B19V and, more importantly, would not detect other new or emerging



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**Table III** - PARV4 DNA findings by PCR in blood donors or plasma-derived medicinal products.

| PARV4 DNA (%) | Subjects of study | Study author and year of publication |
|---|---|---|
| 5.1 | Plasma donations | Fryer JF, 2006[142] |
| a) 8.7<br>b) 4 | a) Healthy donors, febrile patients, IDUs,<br>b) plasma pools | Fryer JF, 2007[130] |
| 16<br>pre-1990 23%<br>1990-2005 2% | Factor VIII concentrates collected from 1974 to 2005 | Fryer JF, 2007[134] |
| 11.5 | HCV positive subjects and/or IDU | Fryer JF, 2007[138] |
| 5.3 | IDU, blood donors negative for HIV, HCV and HBV | Lurcharchaiwong W, 2008[141] |
| 12.4 | Coagulation factor concentrates | Schneider B, 2008[135] |
| a) 1<br>b) 1.2<br>c) 0<br>d) 1.6 | a) Blood donors,<br>b) patients with suspected viral diseases,<br>c) BM/PBSC transplant recipients,<br>d) kidney transplant recipients | Vallerini D, 2008[143] |
| 3.8 | Healthy blood and skin donors, skin donors with dermatological disease | Botto S, 2009[144] |
| 0 | Transfused immunocompetent patients, blood donors | Servant-Delmas A, 2009[145] |
| 1.7 | HCV, HIV, and HBV positive subjects | Tuke PW, 2010[146] |
| 8.6 | Infants (Ghana) | Panning M, 2010[147] |
| 24 | Blood donors | Touinssi M, 2010[148] |
| 24.5 | Haemodialysis patients positive or not for HBV markers, lung transplant recipients | Touinssi M, 2011[149] |
| 26 | Plasma donations | Modrow S, 2011[65] |
| 26.15 | Blood donors | Ma YY, 2012[133] |
| 0<br>(4.76 IgG) | Blood donors | Maple PA, 2013[150] |

PCR: polymerase chain reaction; IDU: intravenous drug users; HIV: human immunodeficiency virus; HCV: hepatitis C virus; HBV: hepatitis B virus; BM: bone marrow; PBSC: peripheral blood stem cell.

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viruses with similar characteristics that could pose a hazard to the users of these products.

The universal screening of donated blood for B19V by NAT-based algorithms is currently carried out in Poland[81], Germany (German Red Cross Centres)[51], Austria[51] and Japan[82]. The detection limit set at $10^5$ IU/mL is undoubtedly contributing to the decrease not only of the viral load in pooled source plasma but also of the frequency of seroconversion or symptomatic infection after treatment with blood products.

The implementation of NAT screening with a much higher sensitivity for B19V is unlikely as it would result in a considerable number of components being discarded, thus jeopardising the capacity of blood systems to ensure self-sufficiency of blood and blood products.

In the near future the transmissibility of B19V by transfusion could be better clarified by taking into account not only the level of B19V in the blood product and its overall transfused dose but also the presence of anti-B19V antibodies, their potency, and titre. Donors with persistent IgG anti-B19V might be considered "B19V-safe" for single-donor blood components[157]. Another strategy currently recommended in the Netherlands is based on the identification of negligible B19V infectivity and the definition of selected indications for the transfusion of a "B19V-safe" blood component (i.e. a blood component donated by a donor in whom "IgG antibodies against B19V have been detected in two separate blood samples, one taken at least six months after the other")[157].

Other factors to be considered are the immune and anti-B19V status of the recipient as well as his/her B19V infection history, which can influence viral persistence. In addition, the approach to high-risk patients requires particular care.

However, as the extent of clinical disease due to transfusion transmission is unknown and reported infrequently, the benefits of (universal) B19V-blood-donor screening may be minimal and, at the moment, not justified, especially in countries with low endemicity[158].

For the immediate future PARV4 is likely to remain under suspicion as a cause of different symptoms in subsets of infected individuals. Continued evaluation of the incidence of PARV4 in treated individuals and disease associations of PARV4 infections is also required to support decision making on whether costly measures such as testing and excluding PARV4-positive donations from fresh blood inventories should be implemented.



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At the moment, the pathological role, clinical relevance, and epidemiology of HBoV1 remain unclear thus making any assessment of its possible role in blood-product safety speculative.

**Keywords:** Parvovirus B19, Parvovirus B19 prevalence, transfusion-transmitted Parvovirus B19, blood product safety, plasma-derived medicinal product.

*The Authors declare no conflicts of interest.*

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