

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}, chikungunya³, babesia⁴, dengue⁵, hepatitis E virus⁶, and variant of Creutzfeldt-Jakob disease⁷.

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 disease⁸. 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 B19V⁹.

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 species¹⁰, is a small non-enveloped DNA virus, discovered in 1975 by Yvonne Cossart in the blood of a healthy blood donor¹¹. 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 nm¹². 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 replication¹² (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 prototype¹³. It largely replaced genotype 2 viruses, which were common in Europe half a century ago¹⁴ and, therefore, has been sporadically found in plasma donations from Europe and North America¹⁵. Genotype 3 has been found predominantly in West Africa (Ghana) thus implying a different and longer evolutionary history, probably rooted in Africa¹⁶. 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 believed¹⁷.

In its infection cycle, the virus links to a specific receptor on the surface of host cells¹⁸ (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

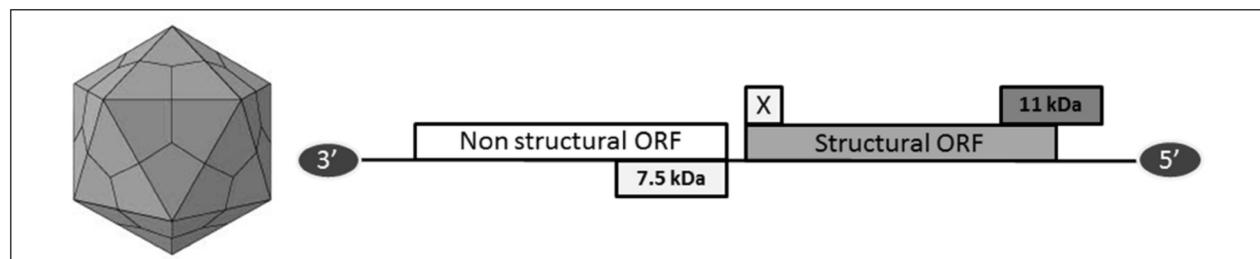


Figure 1 - Schematic representation of Parvovirus B19 icosahedral virion and genome structure.
ORF: open reading Frame; kDa: kiloDaltons; X: Region X.

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 cells¹². Gb4 is also responsible for the phenomenon of B19V haemagglutination¹⁹. Individuals who lack the blood group P antigen on their erythroid cells are not, therefore, susceptible to B19V infection²⁰. The B19V receptor is also present on platelets, cardiac, synovial, renal and thyroid cells as well as on hepatic progenitors⁸. Interestingly, between 1989 and 1992, B19V replication was shown in a primary culture of erythroid cells derived from foetal liver²¹, in haematopoietic progenitor cells from a blood donor²², and in human umbilical cord blood cells²³.

The bone marrow cell tropism of B19V is now well recognised as an erythroid progenitor cell tropism²⁴. 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 crisis²⁰. 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 replication¹⁹.

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-term²⁵.

Parvovirus serology (anti-B19V immunoglobulin [Ig] M and IgG antibodies) can be determined using enzyme-linked immunosorbent assay (ELISA), radioimmunoassay, chemiluminescence or immunofluorescence²⁵.

IgA antibodies are also detectable for a short period and may play a protective role in the respiratory tract²⁶. In addition, long-term B19V-specific IgE antibodies have been found, but their biological functions are unclear²⁷.

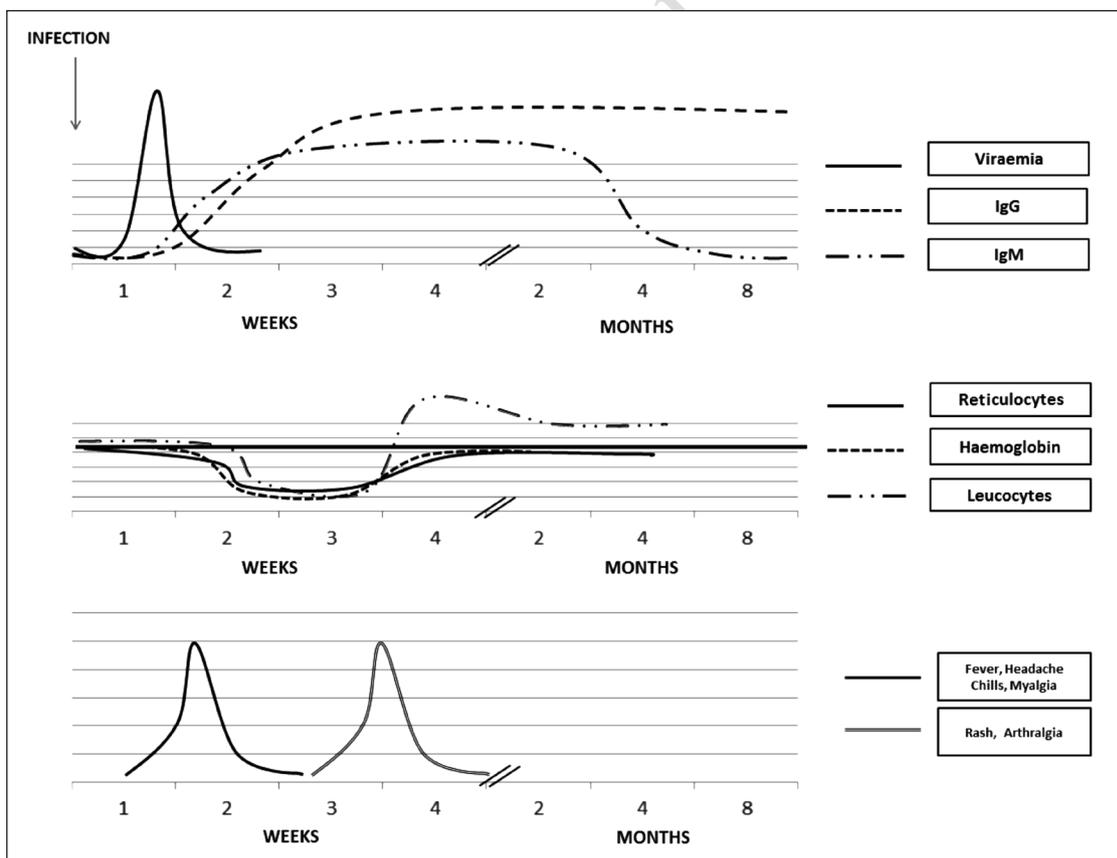


Figure 2 - Clinical, haematological and viral course after B19V infection in immunocompetent subjects. B19V: Parvovirus B19; IgG: immunoglobulin G; IgM: immunoglobulin M.

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 disease²⁵. 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/mL³⁰.

Results of IgM testing are particularly difficult to interpret³¹. 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 result³¹.

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 infection³⁰, but can persist even longer at low levels³². Therefore, B19V DNA, detected by qualitative PCR analysis, is not always indicative of recent infection²⁵. 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 disease³³.

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 host³⁶. 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 crisis³⁷. 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 anaemia³⁴.

Other complications associated with B19V infection include encephalopathy, epilepsy, meningitis, myocarditis, dilated cardiomyopathy, and autoimmune hepatitis³⁴.

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 infections²⁵, or red blood cell transfusion, when necessary³⁹. 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 summer³⁶.

B19V is mainly transmitted through the respiratory route and its DNA is contained in respiratory secretions at the time of viraemia, when infectivity is highest⁴⁰. Transmission in the household, at day care, and in school is common, although the virus can also be transmitted vertically⁴¹ and via blood transfusion and organ/bone marrow transplantation¹⁴.

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 cases⁴¹. 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 failure⁴²⁻⁴⁵. 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 infection⁴⁶.

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 virus⁴⁷, 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 groups⁵⁰.

Transmission via plasma-derived medicinal products can occur because of incomplete physiological clearance of virus, high-level viraemia in acutely infected individuals¹⁴, and the resistance of B19V to most viral inactivation procedures used in the manufacturing of blood-derived products⁵¹⁻⁵⁵.

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 virus⁵⁷⁻⁶². 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 2008⁶⁵. 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 B19V⁶⁶. 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 DNA⁶⁷. The viral load in those samples ranged from 1×10^2 to 1×10^6 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 1×10^2 to 1×10^8 geq/mL⁹.

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 results⁶⁸. 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 seroconversion⁶⁹.

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 nanofiltration⁷⁰. 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 affected⁷⁰. Transfusion transmission of B19V was also reported with solvent/detergent-treated plasma⁵⁴.

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 product¹³. 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 infection⁷¹.

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 derivatives⁷². Similarly, the European Pharmacopoeia has imposed a limit of 10^4 UI/mL of B19V in anti-D immunoglobulins and pooled virus-inactivated plasma⁷³.

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 recipient⁷⁴. 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 irrelevant⁶⁸ while vulnerability to serious B19V-related haematological disorders is dependent on the patients' underlying diseases⁵⁰.

Studies in different countries found B19V DNA in 1% of all blood cell preparations and blood products transfused to patients in a haematology ward⁴⁹, in 0.9% of standard blood components⁴⁹ (in 2% of pooled-plasma products and in 0.7% of single-donor products)⁴⁹, in 0.006% of blood donations⁷⁵, in 0.14% of single-donor blood products⁷⁶, and in 0.16% of plasma samples⁷⁷. 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 DNA⁶⁷. 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 viraemia⁴⁹.

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 Austria⁵¹. 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 immunisation⁸¹. 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 NAT⁸². There has been a subsequent expansion of B19V DNA screening of pools of plasma used to manufacture plasma derivatives in many countries⁸³. 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 products⁸⁴.

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 available⁸³. 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 donors⁸⁵.

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 years¹⁰¹), 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.

Table 1a - 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+)	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+: immunoglobulin G positivity; IgM+: immunoglobulin M positivity; IgG&IgM+: immunoglobulin G and immunoglobulin M positivity; geq/mL: genome equivalents/mL.

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 understood¹²⁸.

PARV4 is a member of the *Primate Tetraparvovirus I* species¹⁰, *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 negative¹²⁹. A related virus variant Parvovirus 5 (PARV5) was identified in plasma pools used in the manufacturing process of plasma-derived medicinal products¹³⁰. 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 syndrome¹³¹; non-parenteral transmission might contribute to its transmission in this area¹³². 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 Asia¹³⁴, 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 explanations¹².

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

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
			-	-	-	0.006 (0: IgG+ 24: IgM+ 6: IgG&IgM+)	Blood donors, plasma pools	Kooistra K, 2010 ¹⁰⁸
	The Netherlands		-	-	-	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	Letaief M, 1997 ¹¹⁷	

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

general, older concentrates were found to be more frequently contaminated with PARV4¹³⁷. This could be due either to differences in population-based hazard of infection over time or to improvements in manufacturing processes¹².

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 patients¹³⁸⁻¹⁴¹. 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 patients¹⁵¹.

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 infections¹⁵². 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

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 b) Haemophilia B: 35.5 c) vWD: 37.9 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 b) Haemophilia B: 83.3 c) Controls: 34.6 IgM was found in 2 patients with haemophilia. 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 b) Haemophilia B: 97 c) vWD: 80 Total 93	Patients with bleeding disorders	Mausser-Bunschoten EP, 1998 ¹²³
IgG: 84 IgM: 0	Patients with bleeding disorders	Aguilar Franco C 1997 ¹²⁴
81.6	Patients with haemophilia	Ragni MV, 1996 ¹²⁵
a) Haemophiliacs: 31 b) Controls: 5	Patients with haemophilic arthritis	Zakrzewska K, 2001 ¹²⁶
a) Haemophiliacs: 55.5 Heated products 93.3 Unheated products b) Healthy donors: 29.3	Patients with haemophilia and healthy donors	Bartolomei Corsi O, 1988 ¹²⁷

B19V: parvovirus B19; vWD: von Willebrand disease; IgG: immunoglobulin G; IgM: immunoglobulin M.

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 diarrhoea¹⁵³. 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 women¹⁵³. 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 seroprevalence of HBoV in adults was 34% for HBoV2, 15% for HBoV3, and 2% for HBoV4¹⁵⁵.

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 pools¹². 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.

Conclusion

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-filtration¹⁵⁶.

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

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 b) 4	a) Healthy donors, febrile patients, IDUs, b) plasma pools	Fryer JF, 2007 ¹³⁰
16 pre-1990 23% 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 b) 1.2 c) 0 d) 1.6	a) Blood donors, b) patients with suspected viral diseases, c) BM/PBSC transplant recipients, 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 (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; IgG: immunoglobulin G.

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 Poland⁸¹, Germany (German Red Cross Centres)⁵¹, Austria⁵¹ and Japan⁸². The detection limit set at 10⁵ 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 components¹⁵⁷. 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 endemicity¹⁵⁸.

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.

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.

References

- 1) Pupella S, Pisani G, Cristiano K, et al. West Nile virus in the transfusion setting with a special focus on Italian preventive measures adopted in 2008-2012 and their impact on blood safety. *Blood Transfus* 2013; **11**: 563-74.
- 2) Grazzini G, Liumbruno GM, Pupella S, et al. West Nile virus in Italy: a further threat to blood safety, a further challenge to the blood system. *Blood Transfus* 2008; **6**: 235-7.
- 3) Liumbruno GM, Calteri D, Petropulacos K, et al. The chikungunya epidemic in Italy and its repercussion on the blood system. *Blood Transfus* 2008; **6**: 199-210.
- 4) Lobo CA, Cursino-Santos JR, Alhassan A, Rodrigues M. Babesia: an emerging infectious threat in transfusion. *PLoS Pathog* 2013; **9**: e1003387.
- 5) Teo D, Ng LC, Lam S. Is dengue a threat to the blood supply? *Transfus Med* 2009; **19**: 66-77.
- 6) Marano G, Vaglio S, Pupella S, et al. Hepatitis E: an old infection with new implications. *Blood Transfus* 2014; doi: 10.2450/2014.0063-14
- 7) Calizzani G, Vaglio S, Vetrugno V, et al. Management of notifications of donors with Creutzfeldt-Jakob disease (post-donation information). *Blood Transfus* 2014; **12**: 22-7.
- 8) Dodd RY. B19: benign or not? *Transfusion* 2011; **51**: 1878-9.
- 9) Alter HJ, Stramer SL, Dodd RY. Emerging infectious diseases that threaten the blood supply. *Semin Hematol* 2007; **44**: 32-41.
- 10) Cotmore SF, Agbandje-McKenna M, Chiorini JA, et al. The family Parvoviridae. *Arch Virol* 2014; **159**: 1239-47.
- 11) Cossart YE, Field AM, Cant B, Widdows D. Parvovirus-like particles in human sera. *Lancet* 1975; **1**: 72-3.
- 12) Norja P, Lassila R, Makris M. Parvovirus transmission by blood products - a cause for concern? *B J Haematol* 2012; **159**: 385-93.
- 13) Blümel J, Burger R, Drost C, et al. Parvovirus B19 - revised. *Transfus Med Hemother* 2010; **37**: 339-50.
- 14) Koppelman MH, Rood IG, Fryer JF, et al. Parvovirus B19 genotype 1 and 2 detection with real-time polymerase chain reaction assays. *Vox Sang* 2007; **93**: 208-15.
- 15) Baylis SA. Standardization of nucleic acid amplification technique (NAT)-based assays for different genotypes of parvovirus B19: a meeting summary. *Vox Sang* 2008; **94**: 74-80.
- 16) Candotti D, Etiz N, Parsyan A, Allain JP. Identification and characterization of persistent human erythrovirus infection in blood donor samples. *J Virol* 2004; **78**: 12169-78.
- 17) Hübschen JM, Mihneva Z, Mentis AF, et al. Phylogenetic analysis of human parvovirus B19 sequences from eleven different countries confirms the predominance of genotype 1 and suggests the spread of genotype 3b. *J Clin Microbiol* 2009; **47**: 3735-8.
- 18) Brown KE, Anderson SM, Young NS. Erythrocyte P antigen: cellular receptor for B19 parvovirus. *Science* 1993; **262**: 114-7.
- 19) Weigel-Kelley KA, Yoder MC, Srivastava A. Recombinant human parvovirus B19 vectors: erythrocyte P antigen is necessary but not sufficient for successful transduction of human hematopoietic cells. *J Virol* 2001; **75**: 4110-6.
- 20) Brown KE, Hibbs JR, Gallinella G, et al. Resistance to parvovirus B19 infection due to lack of virus receptor (erythrocyte P antigen). *N Engl J Med* 1994; **330**: 1192-6.
- 21) Yaegashi N, Shiraishi H, Takeshita T, et al. Propagation of human parvovirus B19 in primary culture of erythroid lineage cells derived from fetal liver. *J Virol* 1989; **63**: 2422-6.
- 22) Schwarz TF, Serke S, Hottentrager B, et al. Replication of parvovirus B19 in hematopoietic progenitor cells generated in vitro from normal human peripheral blood. *J Virol* 1992; **66**: 1273-6.
- 23) Srivastava CH, Zhou S, Munshi NC, et al. Parvovirus B19 replication in human umbilical cord blood cells. *Virology* 1992; **189**: 456-61.
- 24) Takahashi T, Ozawa K, Takahashi K, et al. Susceptibility of human erythropoietic cells to B19 parvovirus in vitro increases with differentiation. *Blood* 1990; **75**: 603-10.
- 25) Corcoran A, Doyle S. Advances in the biology, diagnosis and host-pathogen interactions of parvovirus B19. *J Med Microbiol* 2004; **53**: 459-75.
- 26) Erdman DD, Usher MJ, Tsou C, et al. Human parvovirus B19 specific IgG, IgA, and IgM antibodies and DNA in serum specimens from persons with erythema infectiosum. *J Med Virol* 1991; **35**: 110-5.
- 27) Bluth MH, Norowitz KB, Chice S, et al. Detection of IgE anti parvovirus B19 and increased CD23+ B cells in parvovirus B19 infection: relation to Th2 cytokines. *Clin Immunol* 2003; **108**: 152-8.
- 28) Anderson LJ, Tsou C, Parker RA, et al. Detection of antibodies and antigens of human parvovirus B19 by enzyme-linked immunosorbent assay. *J Clin Microbiol* 1986; **24**: 522-6.
- 29) Kühl U, Pauschinger M, Noutsias M, et al. High prevalence of viral genomes and multiple viral infections in the myocardium of adults with "idiopathic" left ventricular dysfunction. *Circulation* 2005; **111**: 887-93.
- 30) Mouthon L, Guillevin L, Tellier Z. Intravenous immunoglobulins in autoimmune- or parvovirus B19-mediated pure red-cell aplasia. *Autoimmun Rev* 2005; **4**: 264-9.
- 31) Bredl S, Plentz A, Wenzel JJ, et al. False-negative serology in patients with acute parvovirus B19 infection. *J Clin Virol* 2011; **51**: 115-20.
- 32) Cassinotti P, Siegl G. Quantitative evidence for persistence of human parvovirus B19 DNA in an immunocompetent individual. *Eur J Clin Microbiol Infect Dis* 2000; **19**: 886-7.
- 33) Baylis SA, Shah N, Minor PD. Evaluation of different assays for the detection of parvovirus B19 DNA in human plasma. *J Virol Methods* 2004; **121**: 7-16.
- 34) Servey JT, Reamy BV, Hodge J. Clinical presentations of parvovirus B19 infection. *Am Fam Physician* 2007; **75**: 373-7.
- 35) van Beers-Tas MH, Heidema J. Pathogenesis of parvovirus infections in children. *Virol Mycol* 2013; **2**: 1.
- 36) Heegaard ED, Brown KE. Human parvovirus B19. *Clin Microbiol Rev* 2002; **15**: 485-505.
- 37) Exindari M, Chatzidimitriou D, Melidou A, et al. Epidemiological and clinical characteristics of human parvovirus B19 infections during 2006-2009 in Northern Greece. *Hippokratia* 2011; **15**: 157-60.
- 38) Kurtzman GJ, Ozawa K, Cohen B, et al. Chronic bone marrow failure due to persistent B19 parvovirus infection. *N Engl J Med* 1987; **317**: 287-94.
- 39) Liumbruno GM, Bennardello F, Lattanzio A, et al. Recommendations for the transfusion of red blood cells. *Blood Transfus* 2009; **7**: 49-64.
- 40) Brown KE. The expanding range of parvoviruses which infect humans. *Rev Med Virol* 2010; **20**: 231-44.
- 41) Pucetti C, Contoli M, Bonvicini F, et al. Parvovirus B19 in

- pregnancy: possible consequences of vertical transmission. *Prenatal Diagnosis* 2012; **32**: 897-902.
- 42) Eid AJ, Chen SF; AST Infectious Diseases Community of Practice. Human parvovirus B19 in solid organ transplantation. *Am J Transplant* 2013; **13** (Suppl 4): 201-5.
 - 43) Heegaard ED, Laub Petersen B. Parvovirus B19 transmitted by bone marrow. *Br J Haematol* 2000; **111**: 659-61.
 - 44) Carraturo A, Catalani V, Ottaviani D, et al. Parvovirus B19 infection and severe anemia in renal transplant recipients. *Scientific World Journal* 2012; **2012**: 102829.
 - 45) Rahiala J, Koskenvuo M, Norja P, et al. Human parvoviruses B19, PARV4 and bocavirus in pediatric patients with allogeneic hematopoietic SCT. *Bone Marrow Transplant* 2013; **48**: 1308-12.
 - 46) Siegl G, Cassinotti P. Presence and significance of parvovirus B19 in blood and blood products. *Biologicals* 1998; **26**: 89-94.
 - 47) Satake M, Hoshi Y, Taira R, et al. Symptomatic parvovirus B19 infection caused by blood component transfusion. *Transfusion* 2011; **51**: 1887-95.
 - 48) Eis-Hubinger AM, Sasowski U, Brackmann HH. Parvovirus B19 DNA contamination in coagulation factor VIII products. *Thromb Haemostasis* 1999; **81**: 476-7.
 - 49) Laub R, Strengers P. Parvoviruses and blood products. *Pathol Biol* 2002; **50**: 339-48.
 - 50) Plentz A, Hahn J, Knöll A, et al. Exposure of hematologic patients to parvovirus B19 as a contaminant of blood cell preparations and blood products. *Transfusion* 2005; **45**: 1811-5.
 - 51) Schmidt M, Themann A, Drexler C, et al. Blood donor screening for parvovirus B19 in Germany and Austria. *Transfusion* 2007; **47**: 1775-82.
 - 52) Willkommen H, Schmidt I, Lower J. Safety issues for plasma derivatives and benefit from NAT testing. *Biologicals* 1999; **27**: 325-31.
 - 53) Koenigbauer UF, Eastlund T, Day JW. Clinical illness due to parvovirus B19 infection after infusion of solvent/detergent-treated pooled plasma. *Transfusion* 2000; **40**: 1203-6.
 - 54) Liunbruno GM, Franchini M. Solvent/detergent plasma: pharmaceutical characteristics and clinical experience. *J Thromb Thrombolysis* 2015; **39**: 118-28.
 - 55) Burnouf-Radosevich M, Appourchaux P, Huart JJ, et al. Nanofiltration, a new specific virus elimination method applied to high-purity factor IX and factor XI concentrates. *Vox Sang* 1994; **67**: 132-8.
 - 56) Azzi A, Morfini M, Mannucci PM. The transfusion-associated transmission of parvovirus B19. *Transfus Med Rev* 1999; **13**: 194-204.
 - 57) Yee TT, Cohen BJ, Pasi KJ, Lee CA. Transmission of symptomatic parvovirus B19 infection by clotting factor concentrate. *Br J Haematol* 1996; **93**: 457-9.
 - 58) Lefrère JJ, Mariotti M. Use of digoxigenin-labelled probes for the detection of B19 parvovirus DNA in batches of blood products. *Cell Mol Biol* 1995; **41**: 985-8.
 - 59) Lefrère JJ, Mariotti M, de la Croix I, et al. Albumin batches and B19 parvovirus DNA. *Transfusion* 1995; **35**: 389-91.
 - 60) Lefrère JJ, Mariotti M, Thauvin M. B19 parvovirus DNA in solvent/detergent-treated anti-haemophilia concentrates. *Lancet* 1994; **343**: 211-2.
 - 61) Rollag H, Solheim BG, Svennevig JL. Viral safety of blood derivatives by immune neutralization. *Vox Sang* 1998; **74**: 213-7.
 - 62) Saldanha J, Minor P. Detection of human parvovirus B19 DNA in plasma pools and blood products derived from these pools: implications for efficiency and consistency of removal of B19 DNA during manufacture. *Br J Haematol* 1996; **93**: 714-9.
 - 63) Corcioli F, Zakrzewska K, Fanci R, et al. Human parvovirus PARV4 DNA in tissues from adult individuals: a comparison with human parvovirus B19 (B19V). *Virology* 2010; **7**: 272.
 - 64) Geng Y, Wu C-G, Bhattacharyya SP, et al. Parvovirus B19 DNA in factor VIII concentrates: effects of manufacturing procedures and B19 screening by nucleic acid testing. *Transfusion* 2007; **47**: 883-9.
 - 65) Modrow S, Wenzel JJ, Schimanski S, et al. Prevalence of nucleic acid sequences specific for human parvoviruses, hepatitis A and hepatitis E viruses in coagulation factor concentrates. *Vox Sang* 2011; **100**: 351-8.
 - 66) Soucie JM, Monahan PE, Kulkarni R, et al. Evidence for the continued transmission of parvovirus B19 in patients with bleeding disorders treated with plasma-derived factor concentrates. *Transfusion* 2013; **53**: 1143-4.
 - 67) Saldanha J. Validation and standardisation of nucleic acid amplification technology (NAT) assays for the detection of viral contamination of blood and blood products. *J Clin Virol* 2001; **20**: 7-13.
 - 68) Kleinman SH, Glynn SA, Lee TH, et al. National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study-II (NHLBI REDS-II). A linked donor-recipient study to evaluate parvovirus B19 transmission by blood component transfusion. *Blood* 2009; **114**: 3677-83.
 - 69) Klukowska A, Komrska V, Jansen M, Laguna P. Low incidence of factor VIII inhibitors in previously untreated patients during prophylaxis, on-demand treatment and surgical procedures, with Octanate®: interim report from an ongoing prospective clinical study. *Haemophilia* 2011; **17**: 399-406.
 - 70) Mundt JM, Rouse L, Van den Bossche J, Goodrich RP. Chemical and biological mechanisms of pathogen reduction technologies. *Photochem Photobiol* 2014; **90**: 957-64.
 - 71) Goeyvaerts N, Hens N, Ogunjimi B, et al. Estimating infectious disease parameters from data on social contacts and serological status. *J Royal Stat Soc* 2010; **13**: 255.
 - 72) US Food and Drug Administration. Guidance for Industry: Nucleic Acid Testing (NAT) to Reduce the Possible Risk of Parvovirus B19 Transmission by Plasma-Derived Products; 2009. Available at: <http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/blood/ucm071592.htm>. Accessed on 25/06/2014.
 - 73) Council of Europe. Human plasma (pooled and treated for virus inactivation), monograph 1646. In: *European Pharmacopoeia*. 6th ed. Strasbourg: Council of Europe Publishing; 2008.
 - 74) Hourfar MK, Mayr-Wohlfart U, Themann A, et al. Recipients potentially infected with parvovirus B19 by red blood cell products. *Transfusion* 2011; **51**: 129-36.
 - 75) Kooistra K, Mesman HJ, De Waal M, et al. Epidemiology of high-level parvovirus B19 viremia among Dutch blood donors, 2003-2009. *Vox Sang* 2010; **100**: 261-6.
 - 76) Jordan J, Tiangco B, Kiss J, et al. Human parvovirus B19: prevalence of viral DNA in volunteer blood donors and clinical outcomes of transfusion recipients. *Vox Sang* 1998; **75**: 97-102.
 - 77) Broliden K, Tolfvenstam T, Norbeck O. Clinical aspects of parvovirus B19 infection. *J Internal Med* 2006; **260**: 285-304.
 - 78) Petermann R, Piquet Y, Lapeyre M, et al. Detection of B19 parvovirus in plasma pools before solvent-detergent treatment of plasma: AFSSAPS and EFS Aquitaine-Limousin's experience. *Transfus Clin Biol* 2010; **17**: 54-62 [In French].
 - 79) Zhang W, Ke L, Changqing L, et al. Parvovirus B19V DNA contamination in Chinese plasma and plasma derivatives. *J Transl Med* 2012; **10**: 194.
 - 80) Kleinman SH, Glynn SA, Lee TH, et al. National Heart, Lung, Blood Institute Retrovirus Epidemiology Donor Study (REDS-II). Prevalence and quantitation of parvovirus B19 DNA levels in blood donors with a sensitive polymerase chain reaction screening assay. *Transfusion* 2007; **47**: 1756-64.

- 81) Grabarczyk P, Korzeniowska J, Liszewski G et al. Parvovirus B19 DNA testing in Polish blood donors, 2004-2010. *Przegl Epidemiol* 2012; **66**: 7-12.
- 82) Sakata H, Matsubayashi K, Ihara H, et al. Impact of chemiluminescent enzyme immunoassay screening for human parvovirus B19 antigen in Japanese blood donors. *Transfusion* 2013; **53**: 2556-66.
- 83) Koppelman MH, Cuijpers HT, Wessberg S, et al. Multicenter evaluation of a commercial multiplex polymerase chain reaction test for screening plasma donations for parvovirus B19 DNA and hepatitis A virus RNA. *Transfusion* 2012; **52**: 1498-508.
- 84) Weimer T, Streichert S, Watson C, Gröner A. High-titer screening PCR: a successful strategy for reducing the parvovirus B19 load in plasma pools for fractionation. *Transfusion* 2001; **41**: 1500-4.
- 85) Lefrère JJ, Servant-Delmas A, Candotti D, et al. Persistent B19 infection in immunocompetent individuals: implications for transfusion safety. *Blood* 2005; **106**: 2890-5.
- 86) Compston LI, Sarkobie F, Li C, et al. Multiplex real-time PCR for the detection and quantification of latent and persistent viral genomes in cellular or plasma blood fractions. *J Virol Methods* 2008; **151**: 47-54.
- 87) Wasfy S, Nishikawa J, Petric M. Seroprevalence of immunoglobulin G antibody to parvovirus B19 in Ontario. *Can J Infect Dis* 1996; **7**: 313-6.
- 88) Slavov SN, Haddad SK, Silva-Pinto AC, et al. Molecular and phylogenetic analyses of human Parvovirus B19 isolated from Brazilian patients with sickle cell disease and β -thalassemia major and healthy blood donors. *J Med Virol* 2012; **84**: 1652-65.
- 89) Slavov SN, Kashima S, Silva-Pinto AC, Covas DT. Genotyping of Human parvovirus B19 among Brazilian patients with hemoglobinopathies. *Can J Microbiol* 2012; **58**: 200-5.
- 90) Lévicán J, Torres M, Gaggero N, et al. Parvovirus B19 among blood donors from three hospitals in Santiago, Chile. *Rev Med Chil* 2011; **139**: 143-9.
- 91) Gaggero A, Rivera J, Calquín E, et al. Seroprevalence of IgG antibodies against parvovirus B19 among blood donors from Santiago, Chile. *Rev Med Chil* 2007; **135**: 443-8.
- 92) O'Bryan TA, Wright WF. Parvovirus B19 and C-reactive protein in blood bank donors: implications for hygiene hypothesis research. *Lupus* 2010; **19**: 1557-60.
- 93) Doyle S, Kerr S, O'Keeffe G, et al. Detection of parvovirus B19 IgM by antibody capture enzyme immunoassay: receiver operating characteristic analysis. *J Virol Methods* 2000; **90**: 143-52.
- 94) Ke L, He M, Li C, et al. The prevalence of human parvovirus B19 DNA and antibodies in blood donors from four Chinese blood centers. *Transfusion* 2011; **51**: 1909-18.
- 95) Wei Q, Li Y, Wang JW, et al. Prevalence of anti-human parvovirus B19 IgG antibody among blood donors in Jilin province. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi*. 2006; **20**: 60-2.
- 96) Kumar S, Gupta RM, Sen S, et al. Seroprevalence of human parvovirus B19 in healthy blood donors. *Med J Armed Forces India* 2013; **69**: 268-72.
- 97) Kishore J, Srivastava M, Choudhary N. Standardization of B19 IgG ELISA to study the seroepidemiology of parvovirus B19 in North Indian voluntary blood donors. *Asian J Transfus Sci* 2010; **4**: 86-90.
- 98) Ihara T, Furusyo N, Hayashi T, et al. A population-based epidemiological survey of human parvovirus B19 infection: a project of the Kyushu and Okinawa Population Study (KOPS). *Arch Virol* 2013; **158**: 2465-72.
- 99) Matsukura H, Shibata S, Tani Y, et al. Persistent infection by human parvovirus B19 in qualified blood donors. *Transfusion* 2008; **48**: 1036-7.
- 100) Wakamatsu C, Takakura F, Kojima E, et al. Screening of blood donors for human parvovirus B19 and characterization of the results. *Vox Sang* 1999; **76**: 14-21.
- 101) Tsujimura M, Matsushita K, Shiraki H, et al. Human parvovirus B19 infection in blood donors. *Vox Sang* 1995; **69**: 206-12.
- 102) Obeid OE. Molecular and serological assessment of parvovirus B19 infections among sickle cell anemia patients. *J Infect Dev Ctries* 2011; **5**: 535-9.
- 103) Oh DJ, Lee YL, Kang JW, et al. Investigation of the prevalence of human parvovirus B19 DNA in Korean plasmapheresis donors. *Korean J Lab Med* 2010; **30**: 58-64.
- 104) Poovorawan Y, Theamboonlers A, Suandork P, Hirsch P. Prevalence of antibodies to parvovirus B 19 in Thailand. *Southeast Asian J Trop Med Public Health* 2000; **31**: 422-4.
- 105) Thomas I, Di Giambattista M, Gerard C, et al. Prevalence of human erythrovirus B19 DNA in healthy Belgian blood donors and correlation with specific antibodies against structural and non-structural viral proteins. *Vox Sang* 2003; **84**: 300-7.
- 106) Hitzler WE, Runkel S. Prevalence of human parvovirus B19 in blood donors as determined by a haemagglutination assay and verified by the polymerase chain reaction. *Vox Sang* 2002; **82**: 18-23.
- 107) Juhl D, Steppat D, Görg S, Hennig H. Parvovirus b19 infections and blood counts in blood donors. *Transfus Med Hemother* 2014; **41**: 52-9.
- 108) Kooistra K, Mesman HJ, de Waal M, et al. Epidemiology of high-level parvovirus B19 viraemia among Dutch blood donors, 2003-2009. *Vox Sang* 2011; **100**: 261-6.
- 109) Corcoran A, Kerr S, Elliott G, et al. Improved detection of acute parvovirus B19 infection by immunoglobulin M EIA in combination with a novel antigen EIA. *Vox Sang* 2007; **93**: 216-22.
- 110) Zaaier HL, Koppelman MH, Farrington CP. Parvovirus B19 viraemia in Dutch blood donors. *Epidemiol Infect* 2004; **132**: 1161-6.
- 111) Gessoni G, Barin P, Di Natale C, Marchiori G. NAT screening for Parvovirus B19: a feasibility study. *Blood Transfus* 2006; **4**: 67-80.
- 112) Manaresi E, Gallinella G, Morselli Labate AM, et al. Seroprevalence of IgG against conformational and linear capsid antigens of parvovirus B19 in Italian blood donors. *Epidemiol Infect* 2004; **132**: 857-62.
- 113) Henriques I, Monteiro F, Meireles E, et al. Prevalence of parvovirus B19 and hepatitis A virus in Portuguese blood donors. *Transfus Apher Sci* 2005; **33**: 305-9.
- 114) Filatova EV, Zubkova NV, Novikova NA, et al. Detection of parvovirus B19 markers in blood samples of donors. *Zh Mikrobiol Epidemiol Immunobiol* 2010; **5**: 67-70.
- 115) Muñoz S, Alonso MA, Fernández MJ, et al. Seroprevalence versus Parvovirus B19 in blood donors. *Enferm Infecc Microbiol Clin* 1998; **16**: 161-2.
- 116) Mata Rebón M, Bartolomé Husson C, Bernárdez Hermida I. Seroprevalence of anti-human parvovirus B19 antibodies in a sample of blood donors in Galicia. *Enferm Infecc Microbiol Clin* 1998; **16**: 25-7.
- 117) Letaïef M, Vanham G, Boukef K, et al. Higher prevalence of parvovirus B19 in Belgian as compared to Tunisian blood donors: differential implications for prevention of transfusional transmission. *Transfus Sci* 1997; **18**: 523-30.
- 118) Reinheimer C, Allwinn R, Doerr HW, Wittek M. Seroepidemiology of parvovirus B19 in the Frankfurt am Main area, Germany: evaluation of risk factors. *Infection* 2010; **38**: 381-5.
- 119) Langara H, Triquia H, Gouiderb E, et al. Blood-transmitted viral infections among haemophiliacs in Tunisia. *Transfus Clin Biol* 2005; **12**: 301-5 [In French].

- 120) Soucie JM, Siwak EB, Hooper WC, et al.; Universal Data Collection Project Working Group. Human parvovirus B19 in young male patients with hemophilia A: associations with treatment product exposure and joint range-of-motion limitation. *Transfusion* 2004; **44**: 1179-85.
- 121) Brojer E, Grabarczyk P, Łopaciuk S, et al. Prevalence of human parvovirus B19 DNA and IgG/IgM antibodies in Polish haemophilia patients. *Vox Sang* 1999; **77**: 107.
- 122) Canales MA, Pinilla J, Mateos P. Human parvovirus B19 antibodies are less frequent among patients treated with factor IX concentrate inactivated by ultrafiltration: a report from a single Spanish institution. *Vox Sang* 1998; **74**: 260-1.
- 123) Mauser-Bunschoten EP, Zaaier HL, van Drimmelen AA. High prevalence of Parvovirus B19 IgG antibodies among Dutch hemophilia patients. *Vox Sang* 1998; **74**: 225-7.
- 124) Aguilar Franco C, Lucía Cuesta JF, Ferrer Torres J, Omeñaca Teres M. Parvovirus B19 infection in patients with congenital blood coagulation disorders. *Med Clin (Barc)* 1997; **108**: 641-6. Erratum in: *Med Clin (Barc)* 1997; **109**: 5 [In Spanish].
- 125) Ragni MV, Koch WC, Jordan JA. Parvovirus B19 infection in patients with hemophilia. *Transfusion* 1996; **36**: 238-41.
- 126) Zakrzewska K, Azzi A, De Biasi E, et al. Persistence of parvovirus B19 DNA in synovium of patients with haemophilic arthritis. *J Med Virol* 2001; **65**: 402-7.
- 127) Bartolomei Corsi O, Azzi A, Morfini M, et al. Human parvovirus infection in haemophiliacs first infused with treated clotting factor concentrates. *J Med Virol* 1988; **25**: 165-70.
- 128) Manning A, Willey SJ, Bell JE, Simmonds P. Comparison of tissue distribution, persistence, and molecular epidemiology of parvovirus B19 and novel human parvoviruses PARV4 and human bocavirus. *J Infect Dis* 2007; **195**: 1345-52.
- 129) Jones MS, Kapoor A, Lukashov VV, et al. New DNA viruses identified in patients with acute viral infection syndrome. *J Virol* 2005; **79**: 8230-6.
- 130) Fryer JF, Delwart E, Hecht FM, et al. Frequent detection of the parvoviruses, PARV4 and PARV5, in plasma from blood donors and symptomatic individuals. *Transfusion* 2007; **47**: 1054-61.
- 131) Simmonds P, Douglas J, Bestetti G, et al. A third genotype of the human parvovirus PARV4 in sub-Saharan Africa. *J Gen Virol* 2008; **89**: 2299-302.
- 132) Drexler JF, Reber U, Muth D, et al. Human parvovirus 4 in nasal and fecal specimens from children, Ghana. *Emerg Infect Dis* 2012; **18**: 1650-3.
- 133) Ma YY, Guo Y, Zhao X, et al. Human parvovirus PARV4 in plasma pools of Chinese origin. *Vox Sang* 2012; **103**: 183-5.
- 134) Fryer JF, Hubbard AR, Baylis SA. Human parvovirus PARV4 in clotting factor VIII concentrates. *Vox Sang* 2007; **93**: 341-7.
- 135) Schneider B, Fryer JF, Oldenburg J, et al. Frequency of contamination of coagulation factor concentrates with novel human parvovirus PARV4. *Haemophilia* 2008; **14**: 978-86.
- 136) Eis-Hübinger AM, Drexler JF, Reber U, et al. Absence of detection of novel human parvoviruses in German plasma donations. *Transfusion* 2010; **50**: 266-7.
- 137) Baylis SA, Tuke PW, Miyagawa E, Blümel J. Studies on the inactivation of human parvovirus 4. *Transfusion* 2013; **53**: 2585-92.
- 138) Fryer JF, Lucas SB, Padley D, Baylis SA. Parvoviruses PARV4/5 in hepatitis C virus-infected persons. *Emerg Infect Dis* 2007; **13**: 175-6.
- 139) Simmonds P, Manning A, Kenneil R, et al. Parenteral transmission of the novel human parvovirus PARV4. *Emerg Infect Dis* 2007; **13**: 1386-8.
- 140) Longhi E, Bestetti G, Acquaviva V, et al. Human parvovirus 4 in the bone marrow of Italian patients with AIDS. *AIDS* 2007; **21**: 1481-3.
- 141) Lurcharchaiwong W, Chieochansin T, Payungporn S, et al. Parvovirus 4 (PARV4) in serum of intravenous drug users and blood donors. *Infection* 2008; **36**: 488-91.
- 142) Fryer JF, Kapoor A, Minor PD, et al. Novel parvovirus and related variant in human plasma. *Emerg Infect Dis* 2006; **12**: 151-4.
- 143) Vallerini D, Barozzi P, Quadrelli C, et al. Parvoviruses in blood donors and transplant patients, Italy. *Emerg Infect Dis* 2008; **14**: 185-6.
- 144) Botto S, Bergallo M, Sidoti F, et al. Detection of PARV4, genotypes 1 and 2, in healthy and pathological clinical specimens. *New Microbiol* 2009; **32**: 189-92.
- 145) Servant-Delmas A, Laperche S, Mercier M, et al. Human parvovirus 4 in recipients of cellular products and in blood donors: epidemiologic similarity with B19 parvovirus. *Transfusion* 2009; **49**: 1771-3.
- 146) Tuke PW, Parry RP, Appleton H. Parvovirus PARV4 visualization and detection. *J Gen Virol* 2010; **91**: 541-4.
- 147) Panning M, Kobbe R, Vollbach S, et al. Novel human parvovirus 4 genotype 3 in infants, Ghana. *Emerg Infect Dis* 2010; **16**: 1143-6.
- 148) Touinssi M, Brisbarre N, Picard C, et al. Parvovirus 4 in blood donors, France. *Emerg Infect Dis* 2010; **16**: 165-6.
- 149) Touinssi M, Reynaud-Gaubert M, Gomez C, et al. Parvovirus 4 in French in-patients: a study of hemodialysis and lung transplant cohorts. *J Med Virol* 2011; **83**: 717-20.
- 150) Maple PA, Beard S, Parry RP, et al. Testing UK blood donors for exposure to human parvovirus 4 using a time-resolved fluorescence immunoassay to screen sera and Western blot to confirm reactive samples. *Transfusion* 2013; **53**: 2575-84.
- 151) Sharp CP, Lail A, Donfield S, et al. Virologic and clinical features of primary infection with human parvovirus 4 in subjects with hemophilia: frequent transmission by virally inactivated clotting factor concentrates. *Transfusion* 2012; **52**: 1482-9.
- 152) Allander T, Tammi MT, Eriksson M, et al. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci USA* 2005; **102**: 12891-96.
- 153) Jartti T, Hedman K, Jartti L, et al. Human bocavirus-the first 5 years. *Rev Med Virol* 2012; **22**: 46-64.
- 154) Norja P, Hedman L, Kantola K, et al. Occurrence of human bocaviruses and parvovirus 4 in solid tissues. *J Med Virol* 2012; **8**: 1267-73.
- 155) Kantola K, Hedman L, Arthur J, et al. Seroepidemiology of human bocaviruses 1-4. *J Infect Dis* 2011; **204**: 1403-12.
- 156) Di Minno G, Canaro M, Ironside JW, et al. Pathogen safety of long-term treatments for bleeding disorders: still relevant to current practice. *Haematologica* 2013; **98**: 1495-8.
- 157) Groeneveld K, van der Noordaa J. Blood products and parvovirus B19. *Neth J Med* 2003; **61**: 154-6.
- 158) AABB Special Issue: Emerging Infectious Disease Agents and their Potential Threat to Transfusion Safety. Human Parvovirus B19. *Transfusion* 2009; **49**: 107S-9S.

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