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BRIEF REPORT

Prevalence of Parvovirus B19 Viremia Among German Blood Donations and the Relationship to ABO and Rhesus Blood Group Antigens

Katie Healy,[1,2] Linda B. S. Aulin,[3,4] Urban Freij,[5] Marie Ellerstad,[5] Lena Brückle,[6] Helen Hillmering,[5] Tor-Einar Svae,[7] Kristina Broliden,[8] and Rasmus Gustafsson[5,9]

[1]Division of Clinical Diagnostics and Surgery, Department of Dental Medicine, Karolinska Institutet, Stockholm, Sweden; [2]Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden; [3]Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands; [4]Clinical Pharmacy and Biochemistry, Freie Universität Berlin, Berlin, Germany; [5]Octapharma AB, Stockholm, Sweden; [6]Octapharma Plasma GmbH, Langenfeld, Germany; [7]Octapharma Handelsges.m.b.H, Vienna, Austria; [8]Karolinska Institutet, Department of Medicine Solna, Center for Molecular Medicine, Division of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden; and [9]Center for Molecular Medicine, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

Background. Asymptomatic blood donors can transmit human parvovirus B19 (B19V).

Methods. We assessed the B19V prevalence among a large cohort of blood donations collected in Germany during 2015–2018.

Results. In total, 167 123 donations were screened for B19V deoxyribonucleic acid with 22 cases of viremia identified (0.013% positive). Infections peaked at a 4-year interval and the highest number of cases occurred in the summer months. All 22 infections were found in rhesus D-antigen-positive donations, suggesting a protective factor in donors who lack this antigen.

Conclusions. These findings contribute to our understanding of risk factors for B19V infection among central European blood and plasma donors.

Keywords. ABO; b19V; blood donations; blood group; rhesus.

The human erythroparvovirus B19 (B19V) is a small, single-stranded, nonenveloped deoxyribonucleic acid (DNA) virus belonging to the *Parvoviridae* family. The B19V replicates in human progenitor cells, leading to a decline in red blood cells that can cause an accompanying anemia [1]. Although it is primarily considered a respiratory disease, B19V can also be transmitted through contaminated blood components as well as plasma-derived medicinal products [2, 3].

In epidemic seasons, blood and plasma donations with high B19V viral load have been reported, posing a risk for vulnerable groups and B19V-naive individuals alike [4]. For example, B19V infection during pregnancy increases the risk of fetal loss, although the incidence rates are low [5]. It is known that the risk of infection through blood products and plasma-derived medicinal products is correlated to the level of viremia, and the exclusion of plasma manufacturing pools containing B19V DNA levels above 10^4 IU/mL prevents transmission through the latter products [6].

The primary B19V receptor has been identified as the P blood group antigen [7]. Although the P antigen is necessary for B19V binding, it alone is not sufficient for viral entry, which implies that there is an additional surface coreceptor involved [8]. Integrin $\alpha_5\beta_1$ and Ku80 have been suggested as coreceptors, but the low expression on B19V-permissive cells indicates that the exact mechanisms underlying B19V entry remain to be elucidated [9]. More recently, antibody-mediated mechanisms of viral entry have been proposed [10]. However, the exact coreceptor that enables B19V entry, and its subsequent endocytosis, is not yet known.

Given the importance of the P blood group antigens in B19V entry and the well documented importance of ABO and rhesus D-antigen [Rh(D)] blood groups in other virus infections including norovirus, human immunodeficiency virus (HIV), and hepatitis B virus [11], we hypothesized that other blood group antigens may play a role in virus susceptibility. In this study, we aimed to investigate the relationship between ABO blood group, Rh(D), and B19V prevalence in a large cohort of blood donations in Germany. Furthermore, we also investigated B19V infection dynamics among these central European blood and plasma donations over a 4-year period.

METHODS

Ethical Statement

This study was performed within the ethical standards of the Declaration of Helsinki.

All data included in this study were pseudonymized and the original donor identities could not be traced back. Therefore, no ethical permit was required according to the Regional Ethical Review Board in Stockholm.

Human Erythroparvovirus B19 Screening and Blood Group Typing of Plasma Donors

At Octapharma AB (Stockholm, Sweden) plasma donations (n = 167 123) from Germany were dispensed into 96-well

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Correspondence: Rasmus Gustafsson, PhD, Octapharma AB, 11251 Stockholm, Sweden (Rasmus.Gustafsson@ki.se).

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microtiter plates and pooled in minipools using a robot (TECAN) with up to 96 donations. These were further pooled into minipools with 480 donations in each pool. The DNA was extracted from the minipools of 480 donations by an automatic extraction procedure (NucliSens easyMAG; BioMérieux) and screened for the presence of B19V DNA using a proprietary internally controlled fluorescence resonance energy transfer (FRET) real-time quantitative polymerase chain reaction (PCR) assay (LightCycler 2.0; Roche) with primers and probes targeting the B19V nonstructural (NS)1 gene and with a 95% cutoff at 250 International Units (IU)/mL determined using the World Health Organization International Standard for B19V DNA. Before DNA extraction, each sample was spiked with Porcine Parvo virus (PPV) as internal control. Primers and probes targeting the PPV gene NS1 was used, and all samples had to exhibit PPV amplification, as seen with crossing point (Cp) values below a certain level, to be regarded as valid. The Cp value is defined as the cycle at which the fluorescence exceeds background fluorescence and is determined by the software of the PCR instruments. The assay is validated to detect the B19V geno- and subtypes 1, 2, 3a, and 3b. Minipool samples with a Cp value determined by the PCR software, that are lower than the Cp value for the reactive control (titer 10^3 IU/mL), were considered B19V positive. To identify the positive donation, the B19V-positive 480 donations minipools were deconvoluted by testing the appurtenant 96 donations minipools, and the positive 96 pool was in turn further deconvoluted by pooling each row or column from the 96-well microtiter plates to identify the positive donation. The reactive donation(s) were excluded from downstream production of plasma-derived medicinal products. The Cp value was defined as the PCR cycle where the tangent of the amplification curve had the greatest slope. One donation yielded an undetermined PCR result and was excluded from subsequent analyses. Blood typing of plasma donations was determined at Deutsches Rotes Kreuz (Frankfurt, Germany) by agglutination and pattern recognition using the Beckman Coulter PK 7300 ABO- and Rhesus-Assay. All B19V-positive donations were confirmed negative for coinfection with HIV and hepatitis A, B, and C viruses using proprietary, internally controlled reverse-transcriptase PCR assays of minipools of up to 480 donations with 95% limit of detection of 12, 26, 31, or 9.4 IU/mL, respectively. The same pooling strategy as described above for B19V was used. Virus screenings and blood group determinations were part of routine testing for pharmaceutical production, and all laboratory test results were reviewed by a second analyst.

Statistical Analysis

Categorical and variance analyses applied to each comparison are indicated below and performed using GraphPad (v9.0). *P* values of less than .05 were considered statistically significant. The associations between risk of B19V infection and age group, temporal season, sex, and blood group were investigated using multivariate logistic regression analysis ($\alpha = 0.05$) performed in R (v.4.0.5). A preselection step was included, which excluded variables that only had cases in 1 category. Thus, the preselection removed variables for which no odds ratio (OR) could be derived. For variables where B19V was present for more than 1 category, the variables were compared using a category including B19V cases as a reference.

RESULTS

Frequency of Human Erythroparvovirus B19 Infection by Age

In total, 22 of 167 122 donations tested positive for B19V DNA, resulting in an overall positivity rate of 0.013%. The cohort characteristics and blood group-level B19V infection rates are shown in Table 1. The highest frequency of infections (55% of infected individuals) occurred in the 31- to 40-year-old age group, and no cases were detected in blood donations from donors over the age of 50 (Figure 1*A*).

Human Erythroparvovirus B19 Temporal Dynamics

Studies of B19V outbreaks indicate epidemic cycles of symptomatic B19V infection with interepidemic periods of approximately 3–4 years [12]. Although the sampling period of our study was only 4 years, a similar pattern emerged with infection peaks occurring in 2015 and 2018 (Figure 1*B*). To normalize for the low number of cases and variability in total number of donations between the years, we instead expressed the data as "relative frequency". Relative to 2015, there was an 8.5- and 7.5-fold difference in the frequency of infections in 2016 and

Table 1. Summary Demographics for B19V-Screened Plasma Donors Stratified by Blood Type

	A	AB	B	O	Rh(D) Neg	Rh(D) Pos
N (%)	67 736 (40.53%)	27 364 (16.37%)	17 054 (10.20%)	54 968 (32.89%)	28 950 (17.32%)	138 172 (82.68%)
Age (IQR)	35 (26–50)	35 (26–50)	32 (24–48)	32 (25–48)	35 (26–49)	34 (25–49)
Male (%)	40 537 (59.9%)	16 074 (58.74%)	9975 (58.49%)	32 153 (58.49%)	16 503 (57.01%)	82 235 (59.51%)
Infection						
B19V + (%)	14 (0.021%)	2 (0.007%)	0 (0%)	6 (0.011%)	0 (0%)	22 (0.016%)

Abbreviations: IQR, interquartile range; Neg, negative; Pos, positive; Rh(D), rhesus D-antigen.

Notes: Blood group percentages are relative to all individuals in the cohort. Male and B19V-positive percentages are reported relative to individuals having the blood type in the cohort. N is the number of plasma donations that were screened for B19V DNA between the period 2015–2018.

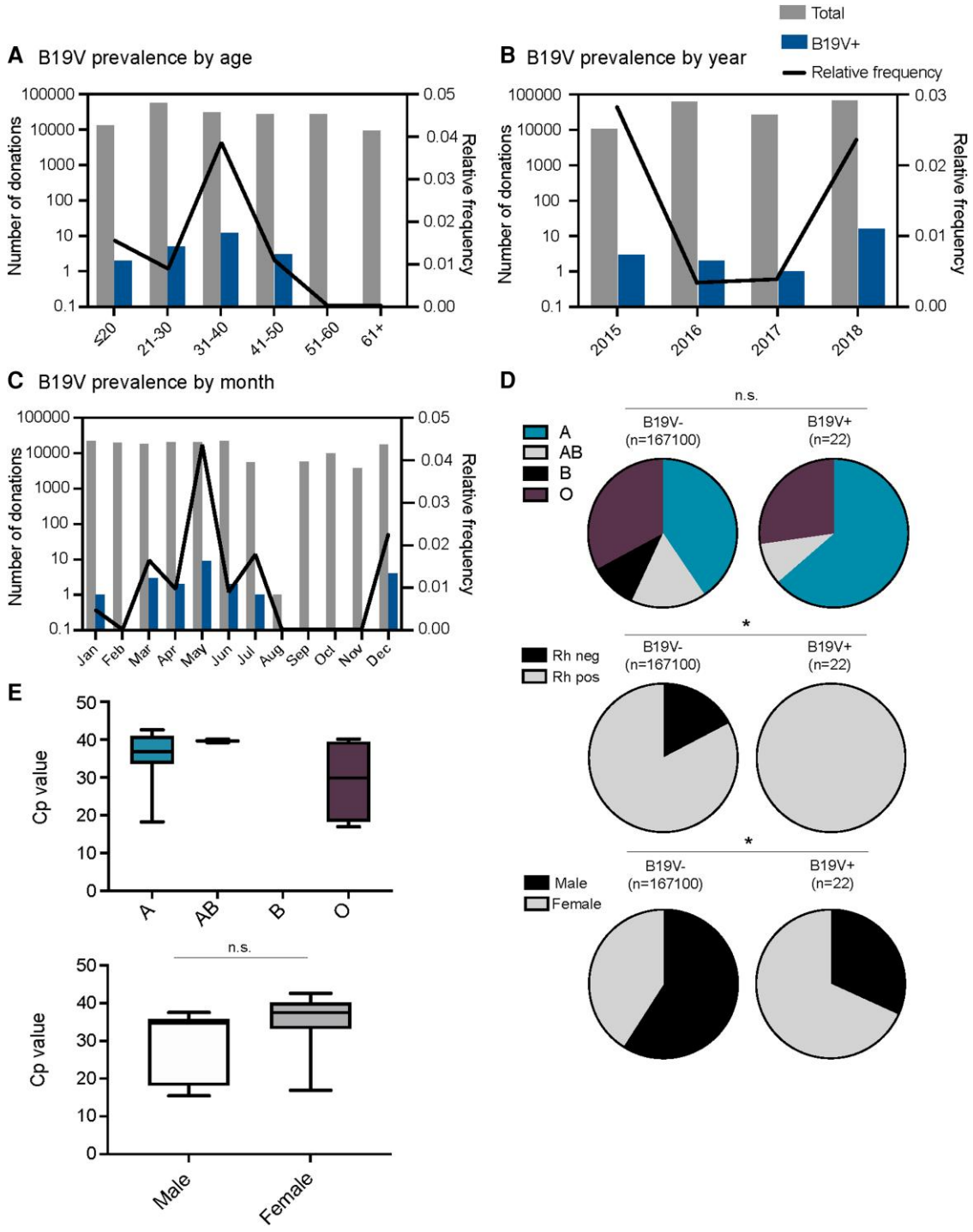

Figure 1. Age, temporal dynamics, and blood group associations with adult human erythroparvovirus B19 (B19V) infection in blood donations. The B19V DNA-positive (pos) plasma donations were plotted relative to the total cohort (black line = relative frequency) by (*A*) age, (*B*) year, and (*C*) month. (*D*) Distribution of B19V infections by sex, Rh(D) factor, and ABO blood group. (*E*) Comparison of viral load, as quantitated by crossing point (Cp) value between ABO blood groups and the sexes (not significant [n.s.]). *, $P < .03$. neg, negative.

2017, respectively. Regarding seasonality, the highest prevalence was found between March and July, with the highest peak in May (41% of infections in May). The lowest prevalence occurred in February, August, September, October, and November, with no viremic donations detected during those months (Figure 1C).

Distribution of Human Erythroparvovirus B19 Infections by Sex and Blood Group

Next, the occurrence of B19V infections by blood group and sex was investigated (Figure 1D). No B19V DNA was detected among blood group B donations, with the highest relative frequency observed in blood group A cohort (n = 14; 0.021%). However, no overall significant differences between the blood groups were found. Despite representing 41% of the donations in the cohort, females accounted for 68% of the B19V infections ($P = .02$; Fisher's exact test). It is remarkable that no B19V cases were detected in Rh(D)-negative individuals, with 100% of cases within the Rh(D)-positive population ($P = .02$; Fisher's exact test). No significant difference in Cp values, which correlate inversely with B19V load, were observed among the ABO blood groups ($P = .57$; Kruskal-Wallis with Dunn's multiple comparisons test) or the sexes ($P = .11$; Mann-Whitney U test) (Figure 1E).

Human Erythroparvovirus B19 Risk Factor Analysis

Multivariate logistic regression analysis was performed to evaluate the categorical variables of ABO blood group, season, age group, and sex as risk factors for B19V infection (Supplementary Table 1). Female sex was identified as the only significant risk ($P < .05$) factor for B19V (OR = 3.11).

DISCUSSION

The present study investigated B19V prevalence and risk factors among a large cohort of German blood donations collected during 2015–2018. The prevalence of B19V found in the present study (0.013%) is at a similar level as a previous report of a Dutch cohort of blood donations (0.006%) [13] but significantly lower compared to previous reports of donations form the Middle East and Northern Africa (1.4%), suggesting that geographical differences occur [14]. Seroprevalence studies of B19V in developed countries have described increased B19V immunoglobulin class G responses by age group, with the highest levels (over 90%) observed in elderly populations [15]. This is in line with our data, where we did not detect any B19V cases in the over 50-year-old age group, suggesting pre-existing immunity among that cohort. It is interesting to note that the highest relative frequency of cases was seen in those aged 31–40 years. We hypothesize that this may correspond to an age group living with small children, and therefore at increased risk of exposure to active infections at home. In addition to pre-existing immunity, less frequent interactions with small children may also contribute to the low prevalence of infection observed in the over 50 years old cohort.

Despite only spanning a 4-year period, an interepidemic pattern was seen with cases spiking in 2015 and 2018. This is in line with what is known about the sporadic epidemiology of B19V outbreaks [12]. The monthly distribution was similar to that observed in previous studies in Germany [16] and the Netherlands [13], with the majority of cases occurring in late winter and spring. Significantly more cases were observed in females than males, which is a similar pattern to previously published data on B19V seroprevalence in Germany [17]. As speculated by Röhrer et al [17], this observation may be due to a larger proportion of females involved with childcare, which is also reinforced by the dominant age profile previously discussed.

It is remarkable that no B19V cases were detected in blood group B or Rh(D)-negative donations, with 100% of cases within the Rh(D)-positive cohort. Rhesus factors are important for immune response, and Rh(D) phenotypes have been described as virus protective, as recently demonstrated in the context of COVID-19 infection [18]. A recent study by Sugrue et al [19] demonstrated enhanced interferon-gamma signaling in Rh(D)-negative males during influenza A infection, which may account for the increased viral resistance seen in these individuals. Another hypothesis is that cell surface bound Rh(D) molecules contributes to the binding and/or integration of the virion into the erythrocyte, constituting a yet unrecognized cellular receptor for B19V. However, it is also noteworthy that blood group B and Rh(D)-negative individuals were underrepresented in this cohort (as in the general population), indicating that sample size may contribute to the observed results. Due to the lack of positive cases in the Rh(D)-negative group, no OR related to the Rh(D) factor could be derived in this study. Nevertheless, future studies involving larger cohorts or infection assays in red blood cells derived from Rh(D)-positive and -negative individuals are warranted to determine whether rhesus antigens contribute to the B19V infectivity. In contrast, ABO blood group did not emerge as a significant risk factor for B19V infection.

CONCLUSIONS

In conclusion, we have described an epidemiological overview of B19V infections in a large cohort of German blood donations collected during 2015–2018. The outcome not only contributes to the current knowledge about the risk for B19V transmission through blood donations and plasma-derived medicinal products, it but also opens exciting novel avenues of research for the potentially protective phenotype of Rh(D) negative against B19V infection and disease.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. R. G. conceived the study design. U. F., M. E., and H. H. collected the data. K. H., L. B. S. A., and R. G. analyzed the data. R. G., K. H., T.-E. S., and K. B. interpreted the data. R. G. supervised the work. R. G. and K. H. wrote the manuscript. All authors reviewed and revised the manuscript critically.

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Potential conflicts of interest. U. F., M. E., L. B., H. H., T.-E. S., and R. G. are all employed at Octapharma, a company specialized in the development and production of human proteins from plasma and cell lines. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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