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DEATH AFTER TRANSFUSION OF PLATELET CONCENTRATE CONTAMINATED WITH KLEBSIELLA PNEUMONIAE

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Background and Objectives: Platelet concentrates (PCs) are the most common source of transfusion-related sepsis. Approximately 1 in 2,000 to 1 in 3,000 units of PCs are contaminated with bacteria originating from the donor's skin or bloodstream. The risk of acquiring a bacterial infection through transfusion is currently 100 to 1,000 times higher than that of transfusion-associated viral infections.
Study Design and Methods: A 58-year-old patient was transfused with an apheresis platelet concentrate (APC) after bone marrow transplantation. Only five minutes into transfusion, the patient was seized with shivering fits. His temperature rose to 39.8°C and blood pressure fell to 60/40 mmHg. The patient went into septic shock, was intubated and treated in an emergency care unit. He eventually died of multiple organ failure 24 days after infection. Bacterial cultures were taken from the recipient's blood samples and also from samples of the original platelet bags.
Results: Klebsiella pneumoniae subspecies pneumoniae were detected in blood samples, as well as in the original PCs. DNA fingerprints authenticated the identity. Capsule type was determined as K62. The bacterial number exceeded 108 CFU/ml 24 hours after spiking with an inoculum of 1-10 CFU/ml, which demonstrated very rapid growth kinetics. LPS concentration also increased to 1 µg/mL. The bacterial strain was neither resistant to common antibiotics nor 70% isopropyl alcohol.
Conclusion: Bacterial infection remains a risk in transfusion medicine. Screening methods or pathogen inactivation methods should add significantly to blood safety.

01.8. Blood Safety - TTD - Parasites

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IMPLEMENTATION OF CHAGAS DISEASE SCREENING IN FRENCH BLOOD DONORS

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Background: Chagas disease is endemic in Latin America. A French government survey showed an increase in the prevalence of the disease in the French Guyana and led to the decision of halting blood collection in this French overseas territory. Thereafter the decision was made to implement trypanosoma cruzi screening in the French blood donors in a targeted at risk population.
Aims: Evaluate the analytical performances of 4 ELISA and an IFA assays and define the criteria of at risk donor eligibility.
Methods: We evaluated the analytical sensitivity, reproducibility and specificity of 4 ELISA kits: Bioelisa Chagas (Biokit, Spain), Chagatck (Lemos, Argentina), Ortho T.cruzi ELISA Test System ' 1 (Ortho, USA) and ELISA Cruzi (BioM rieux, France). The samples were dispensed with an automated pipetting instrument (Microlab AT, Hamilton) and the assays processed according to the manufacturer's instructions, on a microplate processing automated instrument (Summit, Ortho, France). The sensitivity was evaluated with a set of panels: a commercial panel of 15 samples (14 positives and one negative, BBI, USA) collected over 5 countries of Southern America and a 36-sample panel selected from a larger set of positive samples tested with 3 assays by the Sau Paulo blood bank (Brazil): ELISA Bioschile, Abbott, IHA, Biolab Brazil, and IFA Imunocruzi, Biolab Brazil. Reproducibility was assessed by testing a positive sample with a low S/CO ratio (8 replicates per day, during 3 different days). The specificity is evaluated on French blood donors who never had been in Southern America before.
Results: Regarding clinical sensitivity all the positive samples of the BBI panel (14/15) as well as the negative sample were accurately detected by the 4 assays . With regard to the Brazilian donors panel, 12 samples were found non reactive by the 4 assays, as well as the Brazilian assays. Twenty samples were consistently reactive with both Brazilian assays and the 4 evaluated assays. The results of the 4 remaining samples were discrepant. Based on Brazilian results, the sensitivity of the 4 assays is 100 %, no false negative sample was found. Two equivocal samples were found negative by both Biokit ELISA and ELISA Cruzi.The reproducibility was expressed by coefficient of variation on the average of S/CO values of the 24 replicates. It was 6.30 %, 9.30 %, 15.7 % and 22.40 % for Bioelisa Chagas, Chagatek, Ortho Elisa and Elisa Cruzi, respectively. As for the specificity, no false positive sample was found on the first 500 donors tested with the 4 assays. The specificity evaluation is still ongoing, up to 2000 donors tested with each assay.
Conclusions: The 4 evaluated ELISA are suitable for T. cruzi screening. Blood donor screening in France will be performed with 2 simultaneous ELISA tests, one with crude antigens and the other one with recombinant antigens. Positive samples will be confirmed with IFA as well as discrepant samples. Screening will be performed in at risk donors: people born in or from mother born in an endemic area and donors traveling to Southern America, regardless the stay lasting.

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CORRELATION BETWEEN THE SPECIFIC SERUM IMMUNOGLUBULINES (IGE,IGG) WITH EOSINOPHILIA IN CYSTIC HYDATIDOSIS PATIENTS

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Introduction: Cystic hydatidosis is recognized as one of world,s zoonosis. This is caused by an infection with the metacestode stage of Echinococcus granulosus parasite . Diagnosis of this disease is important from both healthy and economical point of view . Therefore , This study was evaluated correlation between the specific Igs (IgE and IgG)with eosinophilia in cystic echinococusis patients who were candidated for surgery .
Materials and Methods: In an analytical correlation from study , the blood samples were obtained from 43 hydatidosis patients who were uder operation . The serum of samples were kept in at ' 20 0c. In orther to detection of eosinophilia percent , the peripheral blood samples were prepared from patients and stained with Wright ' Gimssa . The IFA method was carried out for detection of specific antibodies (IgE and IgG) titer . Correlation between specific immunoglubines (IgE and IgG) with eosinophilia , number and location of cysts removed and existence of germinal layer were recorded from each patients and were analyzed by using Spss.
Results: The increasing of specific antibodies (IgE , IgG) titer were observed in all patients sera but , increasing higher in IgE titeration . Results also indicated that specific antibodies titers were higher than in patients with liver cysts in comparison with lungs and liver kidney

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研究報告の概要

○ジェノタイプ3流行地のヒトエリスロウイルス(パルボウイルス)B19感受性またはB19感染小児受血者に対する輸血の影響

【背景】ヒトエリスロウイルスB19は、血液、血液成分、血漿分画製剤の輸血によって感染し、ほとんどのウイルス不活化法に抵抗性を示す。B19ジェノタイプ3はガーナで蔓延しているが、これに関連した臨床情報は得られていない。

【実験デザインおよび方法】B19ジェノタイプ3の輸血による感染、ならびにウイルス血症を呈した受血者における輸血されたB19抗体の潜在的な影響について評価した。主に急性マラリア性貧血のために輸血を受けた小児を対象に、B19ジェノタイプ3の免疫学的特性を調べた。ジェノタイプ3用に分子学的、血清学的方法を開発し、使用した。

【結果】ガーナの供血者-受血者ペア114組のうち、2件の供血血液にB19 DNAおよび特異的抗体が含まれていたが、感染の証拠は認められなかった。B19免疫グロブリンG(IgG)を含んだ全血は、14名のB19 DNA陽性受血者に輸血された。検出可能レベルのB19IgGを認めた受血者3名は、輸血1～2.3ヵ月後のウイルス血症を排除できなかった。輸血前にVP2に対するIgGを認めなかった受血者10名は、ウイルスを排除できたが、輸血後1～2ヵ月の間B19に対する免疫反応が発現しなかった。検出可能な抗体を生じたのは、少量の特異的IgGを輸血された1名のみであった。

【結論】特異的IgGを伴う低レベルのB19ジェノタイプ3 DNAは、輸血により感染しない。ウイルス排除とB19に対する免疫反応の明らかなダウン・レギュレーションは、輸血された抗体および(または)輸血の免疫変調作用によるウイルス抗原の除去に関連する可能性がある。

報告企業の意見	今後の対応
特異的IgGを伴う低レベルのヒトエリスロウイルス(パルボウイルス)B19ジェノタイプ3 DNAは、輸血により感染せず、ウイルス排除とB19に対する免疫反応の明らかなダウン・レギュレーションは、輸血された抗体および(または)輸血の免疫変調作用によるウイルス抗原の除去に関連する可能性があるとの報告である。	今後も引き続き、ヒトパルボウイルスB19に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

Effects of transfusion on human erythrovirus B19-susceptible or -infected pediatric recipients in a genotype 3–endemic area

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BACKGROUND: Human erythrovirus (parvovirus) B19 is transmitted by transfusion of blood, blood components, and plasma derivatives and is resistant to most viral inactivation methods. B19 genotype 3 is prevalent in Ghana, and no related clinical information is available.
STUDY DESIGN AND METHODS: This study assessed the transmission of B19 genotype 3 by transfusion and the potential effect of transfused B19 antibodies in viremic recipients. Immunological aspects of B19 genotype 3 infection in children mainly transfused for acute malarial anemia were examined. Molecular and serologic methods adapted to genotype 3 were developed and used.
RESULTS: Among 114 donor-recipient pairs from Ghana, two donations contained B19 DNA and specific antibodies, and no evidence of transmission was found. B19 immunoglobulin G (IgG)–containing whole blood was transfused to 14 B19 DNA–positive recipients. Three recipients with detectable levels of IgG to B19 failed to clear viremia 1 to 2.3 months after transfusion. Ten recipients without IgG to VP2 before transfusion cleared the virus but failed to develop an immune response to B19 within 1 to 2 months after transfusion. Only 1 patient who received little specific IgG by transfusion produced detectable antibodies.
CONCLUSION: Low levels of B19 genotype 3 DNA associated with specific IgG are not infectious by transfusion. Viral clearance and apparent down regulation of immune response to B19 may be related to removal of the viral antigens by transfused antibodies and/or immunomodulatory effect of transfusion.

Human erythrovirus (parvovirus) B19 (called B19 in this report) is a 19- to 23-nm-diameter nonenveloped single-stranded DNA virus.[1,2] Its capsid consists of VP2 and VP1 proteins, which are identical, except VP1 has a 227-amino-acid N-terminal extension: the VP1 unique (VP1u) region.[3] Until recently B19 was thought highly genetically conserved but two new genotypes (2 and 3) considered rare were described.[4-7] Far from being rare, genotype 3 was found endemic in Ghana, West Africa.[8] Infection with B19 is common among children, who acquire the infection mainly through personal contact via aerosol or respiratory secretions and develop fifth disease.[9-11] B19 also causes other conditions, such as hydrops fetalis and various forms of anemia (reviewed in Young and Brown[12]).

B19 is transmissible by transfusion of blood, blood components, and plasma derivatives and resistant to most viral inactivation methods.[13-17] Although the seroprevalence of B19 in adult blood donors ranges between 40 and 92 percent depending on age, DNA is detectable in 0.003 to 1.3 percent of donors.[8,14,18-23] The broad range of DNA

ABBREVIATIONS: PBST = phosphate-buffered saline with 0.05 percent Tween 20; S/CO = sample-to-cutoff ratio.

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prevalence reported in the literature might be related to the variations in sensitivity of detection or study populations. Whether or not the association of viral DNA and immunoglobulin G (IgG) prevents infectivity in the transfused blood is a matter of debate. Some data suggest that the low incidence of infection among recipients transfused with B19 DNA–positive components is related to the presence of neutralizing antibodies.[14] Products derived from specific IgG-positive plasma pools containing less than 10^4 geq per mL of B19 DNA were shown to be noninfectious.[13] The potential infectivity of blood components containing concomitant B19 DNA and IgG, however, needs further investigation. This is particularly important, because blood products with viral load below 4×10^3 (geq/mL or IU/mL) but without specific IgG were found infectious.[15,16] In addition, data on transmission, pathogenicity, and immune response to genotypes 2 and 3 are not available.

The present study aimed at assessing the transmission of B19 genotype 3 by blood transfusion and the effect of passive immunity acquired by transfusion on recent B19 infection in young, severely anemic recipients carrying viral DNA. This study was undertaken in a Ghanaian population of pediatric recipients of whole blood collected in an area endemic for B19 genotype 3.

MATERIALS AND METHODS

Study design and sample collection

A prospective study of donor-recipient pairs (n = 114) was conducted from November 2003 to March 2005. The study was approved by the Ethics and Publication Committee of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. Participants or their relatives (when applicable) were informed, and informed consent was obtained. Study population included adult donors and children recipients below 6 years of age, admitted to the Pediatric Emergency Unit of the Komfo Anokye Hospital.

The predominant indication for hospitalization and transfusion was severe anemia related to malaria (93%). At the time of transfusion, hemoglobin levels ranged between 2.8 and 7 g per dL. Each recipient was transfused once with 100 to 200 mL of whole blood negative for antibody to human immunodeficiency virus, hepatitis C virus, and hepatitis B surface antigen[24] according to patient body weight. Posttransfusion samples were collected at home or follow-up visits a mean of 42.5 days after transfusion. Six recipients had a second posttransfusion sample collected approximately 3 months after transfusion.

Detection, quantification, and molecular analysis B19 DNA

Viral DNA was isolated from individual samples as previously described.[8] Detection and quantification of B19 DNA were performed according to a previously described quantitative polymerase chain reaction (PCR).[8] Positive samples or samples reactive in only one replicate were retested in duplicate and confirmed with in-house seminested PCR with a detection limit comparable to the one of the qualitative PCR. The seminested PCR targeted the C-terminal part of the VP1u region and used a PCR system (Expand High Fidelity^{PLUS} PCR system, Roche, Lewes, UK). The first round was performed in 50 µL of reaction with 10 µL of the extracted DNA and 5× reaction buffer with 7.5 mmol per L $MgCl_2$, 200 µmol per L dNTP, 2.5 U of High Fidelity^{PLUS} enzyme blend, 0.4 µmol per L forward, GAPS (5′-ATGGACAGTTATCTGACCACCCCC-3′), and reverse, USTO (5′-GCTGGGGTATTTTTCCGAGGCGT-3′), primers. The second round was performed with the same concentrations of reagents but with 5 µL of the first-round reaction and another forward primer, GAPSi (5′-AGTATTATCTAGTGAAGACTTACACAAGCCTGGG-3′). The cycling conditions for both rounds were denaturation for 5 minutes at 95°C, followed by 35 cycles at 95°C for 30 seconds, 57°C for 40 seconds, and 72°C for 50 seconds, followed by final extension at 72°C for 7 minutes. Only samples positive by seminested PCR were considered confirmed positive. GenBank Accession Numbers of sequenced seminested PCR products are DQ234769, DQ234771, and DQ234774-DQ234789.

Recombinant genotype 3 proteins and immunoassays

IgM to B19 in donors and recipients were tested with parvovirus B19 IgM enzyme immunoassay (EIA; Biotrin International, Dublin, Ireland) according to the manufacturer's instructions.

B19 DNA of genotype 3 (GenBank Accession Number AY582124) previously isolated from a Ghanaian blood donor was used for cloning and expression of B19 genotype 3 proteins in a baculovirus system. The detailed protocol for amplification of VP1 from this sample was described by Parsyan and colleagues.[25] The VP1u region was cloned to baculovirus transfer pBlueBac4.5/V5-His-TOPO vector (Invitrogen, Paisley, UK) and expressed with C-terminal V5/6×His tags in Bac-N-Blue with Sf9 insect cells (Invitrogen), according to the manufacturer's instructions. VP1u was purified by HisTrap HP kit (Amersham Biosciences, Chalfont St. Giles, UK) according to the manufacturer's instructions. VP2 capsids were essentially purified by previously described techniques.[26] In-house EIA with genotype 3 VP2 was developed and used to screen for IgG against nondenatured VP2 capsids (hereafter referred as native VP2). A quantity of 2.5 µg per mL VP2 protein in 100 µL of carbonate-bicarbonate buffer, pH 9.6 (Sigma-Aldrich, St. Louis, MO), was coated on plates (MaxiSorp, Nunc, Roskilde, Denmark) overnight at 4°C. After five washes with 300 µL of phosphate-buffered saline

(PBS), pH 7.4, 0.05 percent Tween 20 (PBST), wells were blocked with 100 µL of PBST plus 4 percent bovine serum albumin (BSA; Sigma-Aldrich) for 2 hours at 37°C. Plates were washed and incubated for 1 hour at 37°C with test plasma diluted 1:100 in PBST plus 1 percent BSA. Following washing as above, plates were incubated at 37°C for 30 minutes with 1:40,000 rabbit anti-human IgG-horseradish peroxidase conjugate (Sigma-Aldrich). After washing, 100 µL of substrate (1-Step Ultra TMB-ELISA, Perbio Science, Cramlington, UK) was added. After 9 minutes, the reaction was stopped by adding 100 µL of 2 mol per L H_2SO_4. Reading was done at 450 nm with a 630-nm reference filter.

Forty Ghanaian children samples previously confirmed negative on repeated testing with the commercial B19 IgG kit (Biotrin International) were used in single well in each plate to determine the assay cutoff, which was defined as the mean OD + 3 standard deviations. Results are expressed as sample-to-cutoff ratio (S/CO). Samples with S/CO of less than 0.9 were considered negative, greater than 1.1 positive, and otherwise equivocal/indeterminate. Averaged S/CO of replicates was used for analysis. Retested samples initially negative or equivocal then equivocal or negative were considered negative. Otherwise, if discrepant with initial test, the retested samples were considered equivocal. Antibody titration was performed with twofold serial dilutions of the sera against NIBSC/WHO international standard for anti-parvovirus B19 (01/602, National Institute for Biological Standards and Control, Potters Bar, UK) calibrated at 77 IU per mL.

For detection of antibodies against denatured (linear epitopes) VP2 and VP1u, in-house Western blotting was developed. Proteins were separated under denaturing conditions by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% for VP2 and 12% for VP1u) and transferred to a nitrocellulose membrane. After blocking with the 10× blocking buffer (Sigma-Aldrich) the membrane was washed three times for 10 minutes with Tris-buffered saline, pH 7.4, and 0.05 percent Tween 20. Strips were incubated for 1 hour with 1:100 (for VP2 WB) or 1:50 (for VP1u WB) dilutions of test samples (the optimal dilution was assessed by a dilution series), washed as above, and incubated for 1 hour with goat anti-human IgG Fc fragment antibodies conjugated to alkaline phosphatase (Sigma-Aldrich). After washing, substrate (5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium, Sigma-Aldrich) was added for 6 to 10 minutes. International standard for B19 antibodies (see above) and IgG-positive and negative-plasma samples from Ghanaian blood donors were used as controls. A recombinant extracellular domain of human platelet glycoprotein VI tagged with V5/6×His or calmodulin were used as negative control for reactivity with V5/6×His tag.

TABLE 1. Epidemiologic data of the donor and recipient populations

Variable	Donors	Recipient children, before transfusion
Number of samples	113*	114
DNA-positive†	2 (1.8)	15 (13.2)
Genotype	3	3
IgG-positive	93 (82.3)	9 (7.9)
IgG-negative	15 (13.3)	104 (91.2)
IgG-indeterminate	5 (4.4)	1 (0.9)
Male/female	1.8‡	1.1 (59/55)
Age range	16-59 years‡	0.13-66 months

* One donation was transfused to two recipients.
† Data are reported as number (%).
‡ Extrapolation from general donor population.[27]

RESULTS

Description of study population

This study included complete sets of 114 donor-recipient pairs with 113 donors and 114 recipients (1 blood unit was transfused to two recipients in the study). The epidemiologic details are shown in Table 1. The mean time interval between pretransfusion and first posttransfusion follow-up samples was 42.5 days (range, 10-86 days).

The prevalence of B19 markers in donated blood was 82.3 percent for IgG and 1.8 percent for viral DNA. The two viremic samples identified also contained B19 antibodies. In contrast, B19 IgG prevalence in children was 7.9 percent (n = 9), with four seropositive samples also containing viral DNA. The prevalence of B19 DNA was 13.2 percent. A majority of viremic samples did not contain IgG to native VP2, but 7 contained IgM. The viral load ranged 9.1×10^1 to 1.7×10^6 IU per mL. All 17 B19 DNA–positive strains were of genotype 3. The sample collection dates of 15 pretransfusion viremic recipients were clustered between December and April 2003 to 2004 or 2004 to 2005 (Fig. 2).

Transfusion of B19 DNA–positive blood

B19 DNA was present in two donations (1.8%). Donation 251 contained 5.8×10^2 IU per mL viral DNA, specific IgG to denatured, and native VP2 but no IgM or anti-VP1u IgG (Table 2). Approximately 200 mL of whole blood from this donor was transfused to a 3-year-old boy without viral or serologic B19 markers. No clinical or laboratory evidence of B19 infection was recorded 33 and 94 days after transfusion.

Donation 1599 contained 2.3×10^5 IU per mL B19 DNA and VP2-specific IgM and IgG (Table 2). Blood was transfused to an 18-month-old girl who had evidence of recent B19 infection. Her plasma contained 8.1×10^2 IU per mL viral DNA and IgM but no VP2- or VP1u-specific

TABLE 2. Summary of donor-recipient pairs that contained human erythrovirus B19 DNA

Donor							Recipient before transfusion							
ID	Age (years)	VL*	IgM*	IgG 1-VP2†	IgG 1-VP2‡	IgG 1-VP1u§	Age (months)	Sex	Diagnosis¶	VL	IgM	IgG n-VP2	IgG 1-VP2	IgG 1-VP1u
251	30	5.8×10^2	–	3.05	3+	–	36	M	MA	–	–	0.37	–	–
1599	28	2.3×10^5	+	1.51	2+	±	18	F	MA	8.1×10^2	+	0.32	–	–
227	17	–	–	3.28	3+	±	24	M	MA	2.9×10^5	+	3.32	2+	–
1579	31	–	±	2.33	2+	–	30	M	MA	9.6×10^3	–	3.84	2+	2+
416	52	–	–	1.56	2+	–	36	F	MA	1.7×10^6	+	5.60	3+	3+
748	23	–	–	3.20	3+	±	60	F	MA	1.2×10^6	+	4.24	3+	–
1589	44	–	–	3.18	–	–	5	M	MA	1.7×10^2	+	0.57	–	–
1598	28	–	–	1.85	2+	–	4	F	MA	1.9×10^3	+	0.52	–	–
223	45	–	–	1.30	1+	1+	18	F	MA	6.0×10^5	+	0.37	–	–
1471	19	–	–	1.21	2+	–	60	M	MA	9.1×10^1	±	0.89	1+	–
1597	25	–	–	2.63	3+	–	36	M	SC	8.0×10^3	–	0.92	3+	–
1621	19	–	–	2.83	3+	1+	9	M	MA	2.1×10^5	–	0.44	2+	–
1623	20	–	–	3.80	3+	–	36	M	MA	5.2×10^2	–	0.23	–	–
693	35	–	–	2.64	3+	±	9	M	BL	1.3×10^4	–	0.21	–	–
234	20	–	–	1.92	2+	3+	9	F	MA	4.6×10^4	–	0.25	–	–
1604	18	–	–	0.74	1+	±	30	F	MA	4.5×10^2	–	0.67	–	–

* Viral load expressed in IU/mL.
† IgG n-VP2 = IgG against VP2 by EIA (native conformation); samples with S/CO below 0.9 are considered negative, over 1.1 positive, and 0.9 to 1.1 indeterminate.
‡ IgG I-VP2 = IgG against VP2 by Western blotting (denatured, linear epitopes); for Western blotting 1+ = weak/medium reactivity, 2+ = strong, 3+ = very strong, ± = equivocal.
§ IgG I-VP1u = IgG against VP1u by Western blotting (denatured, linear epitopes).
MA = malaria; SC = sickle cell disease; BL = bleeding disorder of unknown etiology.
¶ PT2 was available in four recipients.
NA = not available; + = positive; ± = equivocal/indeterminate; – = negative.

IgG. Seventy-two days after transfusion, no B19 DNA or specific IgM was detectable but strong IgG response to VP2 and VP1u was found.

Transfusion of B19 IgG–containing blood to B19 DNA–positive recipients

Before transfusion, 15 recipients had detectable B19 DNA in plasma (Table 2). Seven of these children samples contained specific IgM and 3 of them were also positive for B19 IgG. Thus at least 7 of 15 children had evidence of recent B19 infection indicated by the association of viral DNA and specific IgM. Four recipients (1623, 693, 234, and 1604) carried only viral DNA without serologic markers of B19 infection before transfusion. IgG to denatured and native VP2 was detectable in 12 of 14 donor samples. Specific IgG titers in 10 donors in this group ranged between 35 and 76 IU per mL. At a mean of 1.5 months after transfusion, three recipients remained viremic, although at lower levels, and the other 11 cleared B19 DNA (Table 2).

The blood received by the three recipients who remained viremic after transfusion (227, 416, and 1579) contained comparable IgG reactivity to B19 (Table 2). After transfusion, viremia slightly decreased, and all patients either further developed or maintained IgG reactivity to VP1u. IgG response to native VP2 was also retained, but reactivity to denatured VP2 either decreased or became undetectable.

The second group of 11 recipients who were viremic before transfusion no longer carried B19 DNA in the post-transfusion samples. One recipient (748) had high viral load, specific IgM, and relatively strong IgG reactivity to denatured and native VP2. Seven recipients had no IgG to B19 and 3 carried only IgG to denatured VP2. After transfusion, no evidence of development of a specific antibody response to B19 was observed, except for Recipient 1604 who produced significant levels of all types of IgG to VP2. The blood transfused to this patient contained very low levels of IgG to denatured but not to native VP2. In two recipients (234 and 223), who had a second sampling 3 months after transfusion, the serologic pattern remained unchanged. In three recipients (748, 1621, and 1597), who had IgG to VP2 pretransfusion, the reactivity either decreased or became undetectable.

Serology in B19 DNA–negative pairs

Ninety-eight donor-recipient pairs who were B19 DNA–negative in both donor and pretransfusion recipient samples were screened for the presence of anti-B19 IgG by EIA to study the passive transfer of B19 antibodies in the absence of B19 DNA. IgG to VP2 was present in 78 of 97 donors (80.4%) and 5 of 98 children (5.1%) before transfusion. After transfusion, all of anti-B19 IgG–negative recipients transfused with the anti-B19–positive blood remained negative, suggesting that on average 1.3 months

Recipient after transfusion 1 (PT1)						Recipient after transfusion 2 (PT2)					
Days PT1	VL	IgM	IgG n-VP2	IgG 1-VP2	IgG 1-VP1u	Days PT2	VL	IgM	IgG n-VP2	IgG	IgG 1-VP1u
33	–	–	0.19	–	–	94	–	–	0.25	–	–
72	–	–	2.22	2+	2+						
37	4.5×10^3	+	3.98	1+	±	69	6.9×10^3	–	4.27	1+	3+
35	5.1×10^2	–	3.79	–	2+						
35	1.5×10^4	–	4.49	2+	3+						
51	–	–	0.38	±	–						
NA	–	–	0.39	–	–						
47	–	–	0.24	–	–						
38	–	–	0.36	–	–	99	–	–	0.33	–	–
65	–	–	0.30	1+	–						
49	–	–	0.73	2+	–						
NA	–	–	0.49	–	–						
62	–	–	0.25	–	–						
54	–	–	0.38	–	–						
37	–	–	0.24	–	±	97	–	–	0.23	–	–
68	–	–	2.25	2+	±						

after transfusion, passively transfused IgG to B19 was undetectable. This result did not differ from data obtained in DNA-positive pairs (Table 2). To test the hypothesis that relatively low antibody titer and antibody $t_{1/2}$ were responsible for this observation, four medium to high IgG S/CO samples from DNA-negative and -positive recipients before transfusion were matched by age and S/CO and titrated by limiting dilution. Considering the $t_{1/2}$ of transfused specific IgG in samples tested on average 1.3 months after transfusion, and the dilution factor in the recipients' blood stream (4-8 times), a negative result was predicted and observed.

DISCUSSION

This study examined the effects of transfusion on B19 infection in a genotype 3–endemic area. Based on the sequence analysis of the seminested GAPSi-USTO products, it confirms previous data indicating that B19 genotype 3 was prevalent in Ghana (Fig. 1).[8] The vast majority of children received transfusion for treatment of severe anemia related to malaria, a major indication of pediatric transfusion in Africa.[26,29] Both malaria and B19 are etiologic agents of anemia as they target erythroid cells (reviewed Young and Brown[12] and Menendez et al.[30]), and their coexistence might exacerbate anemia, as previously suggested.[31,32] The possibility that this exacerbation could have increased the likelihood that patients sought medical care because of such association has been examined. In one study, a hospital-based control group of children with median age 10.1 years (range, 2 months-16 years) had 7 percent prevalence of B19 DNA.[33] In our population with a median age of 18 months, 13.2 percent of patients were viremic and more than 90 percent had acute malaria. Some degree of malnutrition is usually observed in approximately one-third of the pediatric transfusion recipients in Kumasi. It is possible, if not likely, that the association of primary infection with both B19 and malarial parasites accompanied by malnutrition increased the severity of anemia and the likelihood of hospitalization.

In temperate countries B19 genotype 1 infection is seasonally distributed with the peak of outbreaks ranging from winter to spring.[9-11,18,20] B19 genotype 3 DNA was detected in Ghana between December and April (Fig. 2), which corresponds to the dry season, during which transmissions by aerosol might be increased by a high level of dust particles and resulting coughing. Clustering of young children as in day care settings or domestic playgroups may contribute to spread.

In the adult donor population, 82.3 percent carried B19 IgG indicating recovered infection. Reported seroprevalence in donors ranges between 40 and 92 percent depending on age and country.[8,18-22] The 1.8 percent prevalence of B19 genotype 3 DNA is consistent with previously reported data from Ghanaian blood donors (Table 1).[8] No transfusion-related infection was documented after transfusion of 200 mL blood containing 580 IU per mL B19 DNA and IgG (Table 2). This anecdotal case is consistent with previous reports suggesting that, in the presence of neutralizing antibodies, the risk of transmission by transfusion of blood products containing relatively low level of viral DNA is negligible.[13,14] This conclusion seems applicable to this case of genotype 3 infection. The response of B19 viremic children who received a transfusion from donors carrying IgG antibodies to this virus was heterogeneous. The major feature of a first group of three recipients was the presence of IgG to both denatured and native VP2 at the time of transfusion and the absence of viral clearance approximately 1 or 2 months after transfusion. Despite the presence of spe-

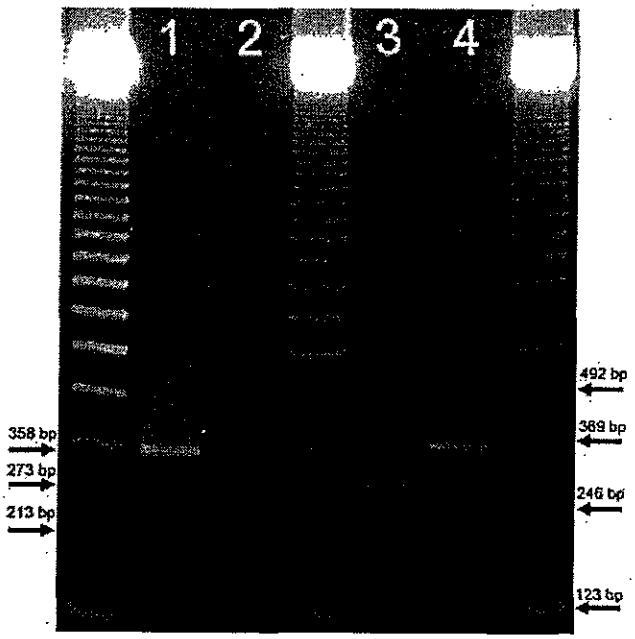

Fig. 1. VP1u amplicons (358 bp) were subjected to *DraI* restriction endonuclease (New England Biolabs, Hitchin, UK) digestion and electrophoresed on 1 to 1.5 percent agarose gels. The expected sizes of digested fragments for genotype 1 are of 213, 85, and 60 bp and for the genotypes 2 and 3 are 273 and 85 bp. To distinguish between G2 and G3, amplicons were sequenced. Lanes 1 and 4 = 358-bp second-round undigested PCR products for B19 genotypes 1 and 3, respectively; Lane 2 = restriction digest of B19 genotype 1 (NIBSC/WHO International Standard), 213-bp product; Lane 3 = restriction digest of B19 genotype 3 (Recipient 416 after transfusion), 273-bp product. The molecular weight of restriction digest fragments are indicated by arrows on the left and of molecular weight markers (123-bp DNA ladder [Invitrogen]) on the right-hand side.

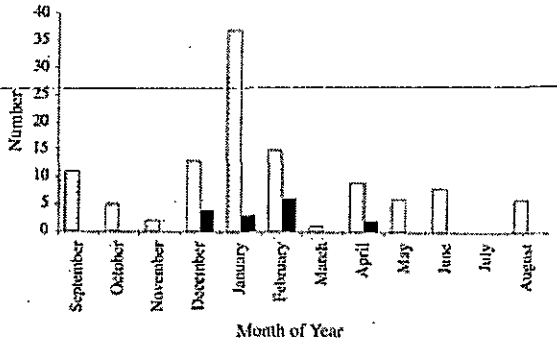

Fig. 2. The month of collection of B19 DNA–negative samples is shown in open bars (n = 113). (■) Time of collection of samples from B19-positive (presumably recently infected) recipients (n = 15).

cific IgG in the recipient and the transfusion of IgG to B19, viremia persisted. This unexpected outcome might be explained by a competition between recipients' and donors' antibodies for the viral antigens. It is hypothesized that at the initial stages of infection with B19 (when viremia is not yet cleared) the neutralizing capacity of antibodies, particularly when produced by young children, might not be very efficient, despite their ability to bind to antigens at the viral surface. Antibodies with binding but poor neutralizing properties were described.[34-36] In this situation, antibodies provided by donor plasma with presumably higher neutralizing capacity might not be able to access neutralizing epitopes on the virions already complexed with host IgG.

The second group of 11 viremic recipients who received specific IgG from transfused blood cleared the virus after transfusion. Clearance of viremia and apparent down regulation of the patients' immune response could be explained by passively transferred immunity to B19. The mechanical clearance of viral particles and antigens from circulation by the passively transfused donor antibodies may have aborted further development of the immune response. The dose of IgG received in 100 to 200 mL of transfused Ghanaian whole blood is considerable, because Ghanaian donors carry 12 to 30 mg per mL IgG (Candotti et al.[8] and unpublished data), ranging between 0.1 and 0.6 g per kg. The role of neutralizing donor IgG in the observed clearance of viremia is indirectly supported by data from Recipient 1604 who, while receiving blood containing no IgG against native VP2 and the lowest reactivity of IgG to denatured VP2, cleared the virus almost 2 months after transfusion and developed a strong IgG response against VP2.

There are, however, alternative or contributing (to the antigen clearance) mechanisms that could be potentially involved in down regulation of the immune response to B19, such as a down regulatory effect of transfusion through a modulatory effect of transfused IgG[37] or other factors present in transfused blood;[38] massive malarial hemolysis and anemia causing temporary blockade or dysregulation of the immune system due to release of cytokines and increased reticuloendothelial activity.[30,39,40] We did not observe clearance of viremia associated to the presence of anti-VP1u, which are considered by some authors the major source of neutralizing activity.[36,41] The presence of neutralizing epitopes in VP2 has been described[34,42] and is corroborated by observations in this study suggesting that anti-VP2 response alone might be sufficient to clear viremia. The significance of antibodies to linear or to native VP2 epitopes deserves some attention. The presence of IgG to linear epitopes of VP2 is considered an indicator of active, recent, or persistent infection.[43-45] The data presented here in children are compatible with this suggestion and support findings indicating that responses to linear epitopes decrease or