Maternal-Fetal Transmission of Human Parvovirus B19 Genotype 3

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Plasma samples obtained at delivery from 885 pregnant Ghanaian women were tested for human parvovirus B19 DNA and B19-specific antibodies. Maternal-fetal transmission was evaluated by testing paired maternal plasma and umbilical cord blood samples, as well as newborn whole-blood samples when they were available. The B19 DNA seroprevalence rate in women was 1.8% (94% had genotype 3 strains), and the immunoglobulin G (IgG) seroprevalence rate in women was 81%. Two of 3 cases of primary maternal B19 infection resulted in fetal transmission. Coexistence of B19 DNA and B19-specific IgG (persistence) was detected in 13 women (1.5%), but no transmission of the virus was observed. Contrary to the situation in pregnant women with primary B19 infection and high viral loads, pregnant women with low viral loads and B19-specific IgG do not appear to be vertically infectious.

Human parvovirus B19 is a common human pathogen that causes mild illness and more serious clinical disorders, as well as complications during pregnancy [1, 2]. Three genotypes have been described recently, and genotype 3, which is rare in Europe, is dominant in Western Africa [3, 4].

In Western countries, 30%–40% of childbearing-age women are susceptible to B19 infection, and intrauterine infection rates range from 24% to 33% [2]. However, most studies that have been performed thus far appear to be biased, because they examined maternal-fetal transmission in retrospective analyses of adverse pregnancy outcomes and in women who were clinically suspected of having primary B19 infection. It may be difficult to identify an acute B19 infection in mostly asymptomatic, recently infected adults [5]. In addition, in newborns surviving fetal infection, persistent asymptomatic infection has been reported [1, 2, 6]. In the absence of systematic surveys, the overall rate of B19 maternal-fetal transmission may be underestimated.

Recent studies using highly sensitive viral nucleic acid testing methods have shown that B19 DNA frequently persists at low levels in immunocompetent individuals for long periods despite the presence of detectable B19-specific IgG [4, 7]. The infectivity of persistent B19 DNA remains to be established. In addition, maternal-fetal transmission has been studied only in women infected with genotype 1 strains. To date, only a few genotype 2 and genotype 3 strains have been characterized in clinical studies [3]. In the present study, we investigated the rate of B19 genotype 3 maternal-fetal transmission by examining paired maternal plasma and umbilical cord blood samples, as well as (when possible) newborn whole-blood samples.

Patients and methods. Plasma samples (coded M) and corresponding cord blood samples (coded C) were collected from pregnant women at delivery in the Department of Obstetrics and Gynaecology, Komfo Anokye Teaching Hospital, Kumasi, Ghana. When possible, newborn whole-blood samples (coded B) were collected 2 weeks after birth. This study was approved by the committee on human research publication and ethics of the University of Science and Technology School of Medical Sciences, Kumasi, Ghana. Informed consent was obtained from the participating women, who presented with no clinical symptoms suggestive of parvovirus-related illness.

Viral nucleic acids were purified as described elsewhere [4]. A standardized amount of human T cell lymphotropic virus I (HTLV-I) was spiked in every sample before purification and was used as an internal control for the entire procedure. B19 DNA was detected using a multiplex real-time quantitative (Q) polymerase chain reaction (PCR) assay that simultaneously detected and identified hepatitis B virus (HBV), human herpesvirus 8 (HHV-8), and B19 DNA. Amplification was performed using the Mx3000P Multiplex Quantitative PCR System with the Brilliant QPCR Core reagent kit (Stratagene). The amplification reaction contained 1× buffer; 5 mmol/L MgCl2; 1.6 mmol/L dNTPs; 0.9 μmol/L HBV primers; 0.8 μmol/L HHV-8 primers; 0.1 μmol/L B19 primers; 0.4 μmol/L HTLV-I primers; 0.2 μmol/L each HBV, HHV-8, and HTLV-I fluorogenic probes; 0.1 μmol/L B19 fluorogenic probe; 2.5 U of SureStart Taq polymerase; and 10 μL of template DNA. After an initial incubation at 95°C for 10 min, the reaction conditions...
were 45 cycles of 1 min at 60°C and 30 s at 95°C. The B19-specific primers (B19F and B19R) and probe (B19P) have been described elsewhere [4]. The 95% detection limit for B19 DNA was 50 IU/mL, and the 50% detection limit was 25 IU/mL (1 IU:2 genome equivalents [8]). B19 DNA was quantified using a single virus real-time QPCR assay [4].

A positive result for B19 DNA was confirmed using semi-nested PCR with the outer primers GAPS and USTO and the inner primer GAPSI, as described elsewhere [9]. The sensitivity of the assay was 50 IU/mL, although a lower viral load was occasionally detected. Phylogenetic analysis of the sequenced 324-bp product was used to determine the genotype [4].

Anti-VP2 IgM was detected with an EIA from Biotrin. Although an IgG EIA from Biotrin recognized antibodies to genotype 1 and 3 strains equally well, it was not suitable to test adult Ghanaian samples [9]; therefore, an in-house EIA that included a recombinant VP2 protein of a genotype 3 strain as the capsid antigen was used for the detection of anti-B19 IgG. Results were expressed as the sample-to-cutoff ratio (S:CO). Equivocal results (defined as 0.9 < S:CO ≤ 1.1) were resolved with a blocking assay, which consisted of incubation of the samples with 15 μg/mL VP2 genotype 3 capsid antigen for 1 h at 37°C and retesting with the standard EIA. A true positive result was defined as a decrease of >50% in the optical density.

Results. Samples from 177 women were randomly selected irrespective of their viral DNA status and were tested for the presence of IgG against a recombinant VP2 protein of a B19 genotype 3 strain; 144 samples (81%) were reactive. A multiplex QPCR assay and a confirmatory seminested PCR assay found that 16 (1.8%) of 885 samples contained B19 DNA, with a viral load ranging from 46 to 3.63 × 10^5 IU/mL (median, 1310 IU/mL) (table 1). Phylogenetic analysis of 324-bp sequences of the VP1–unique (VP1u) region showed that 1 (6%) and 15 (94%) of the 16 Ghanaian B19 sequences clustered with the genotype 1 and genotype 3 reference sequence, respectively (table 1). Extending the phylogenetic analysis to 863-bp sequences, including the nonstructural protein 1 (NS1)–VP1u junction, confirmed these results (data not shown).

The presence of B19 DNA was investigated in cord blood samples collected at delivery from the 16 B19 DNA–positive women and in the 4 available corresponding newborn whole-blood samples (1655B, 1964B, 2196B, and 2409B) that were collected 2 weeks after birth. Two (12.5%) of the 16 cord blood samples (1655C and 2005C) were repeatedly positive for B19 DNA. The viral load was 1.09 × 10^3 and 2.11 × 10^6 IU/mL, respectively. Sample 1964B was positive for B19 DNA, with a viral load of 1.69 × 10^7 IU/mL (table 1). The VP1u and NS1–VP1u regions were amplified and sequenced from corresponding mother–newborn samples (1964M, 1964C, and 2196B). Within each cluster, the sequences were identical. The women were therefore considered to be transmitting B19, and semiquantitative serological studies were conducted. Samples 1964M and 2005M were reactive for both anti-VP2 IgM and anti-VP2 IgG (both titers, 1:1000), which

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>B19 genotype</th>
<th>Maternal plasma</th>
<th>Cord blood</th>
<th>Newborn whole-blood, viral load, IU/mL*</th>
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* ND, not done.

Table 1. Virological and serological parameters in maternal plasma, cord blood, and newborn whole-blood samples.
suggested that there was recent infection. A similar titer of anti-VP2 IgG was detected in the paired cord blood samples. Sample 2005C was reactive for anti-VP2 IgM (titer, 1:1000), which suggested that there was ongoing primary infection of the fetus.

The 14 nontransmitting mothers, who were characterized by their B19 DNA–negative cord blood samples and the B19 DNA–negative whole-blood samples from their newborns, had lower viral loads (median, $1.18 \times 10^5$ IU/mL [range, 46–3.63 $\times 10^6$ IU/mL]) than did transmitting mothers with samples M1964 ($1.14 \times 10^5$ IU/mL) and M2005 ($1.21 \times 10^5$ IU/mL) ($P = .057$). No detectable B19 DNA was observed in the cord blood and newborn whole-blood samples that corresponded with sample 1655M, which had the highest viral load ($3.63 \times 10^4$ IU/mL) and was the only sample from the group of nontransmitting mothers to contain anti-VP2 IgM. The IgM titer in sample 1655M (1:100) was lower than that in the samples from the 2 transmitting mothers (both titers, 1:1000).

No significant difference in IgG titers was observed between samples from the transmitting and nontransmitting groups of mothers. However, one nontransmitting mother had a 2-log higher anti-VP2 IgG titer in her sample (M1655), compared with that in samples M1964 and M2005 from transmitting mothers.

Analysis of the VP1u region showed that there were 1–6 aa differences spread over the entire protein between transmitted and untransmitted viruses. Identical hydrophilicity and antigenicity profiles were observed in the B19 strains, regardless of the maternal-fetal infection outcome.

**Discussion.** The 81% anti-VP2 B19 IgG seroprevalence rate that we observed in a population of pregnant Ghanaian women was higher than that reported in pregnant women from Western countries (35%–66%) and South Africa (25%) but was consistent with the 82% and 83% seroprevalence rate that was found in pregnant women from Eritrea and in Ghanaian blood donors, respectively [2, 4, 7, 10, 11]. These differences may be related, in part, to epidemiological features that are characteristic of many parts of Africa. However, a similar seroprevalence rate (81%) was reported during a nonepidemic period in an area of Sweden [12].

In our study of pregnant Ghanaian women, the B19 DNA seroprevalence rate was 1.8%. This finding is consistent with the 1.3% seroprevalence rate that was previously observed in blood donors, 70% of whom were males, from the same area [4]. However, it is higher than the reported 0.8% seroprevalence rate in 500 pregnant women from France, although the difference is not significant ($P = .13$) and may reflect differences in the sensitivity of the molecular assays that were used rather than an epidemiological difference [7]. In that study, B19 infection was assessed on the basis of genomic screening alone. Because B19 infection is often asymptomatic and most infections do not result in adverse effects for the fetus, it is possible that an early infection that was cleared by the maternal immune response would be missed when this testing strategy is used. Viral clearance has been recently described as a slower process than had been previously envisaged [13]. Therefore, there is a low likelihood that true B19 infection is missed when DNA testing is used in pregnant women.

Our molecular analysis confirmed that B19 genotype 3 was prevalent (94%) in Ghana [4]. A single genotype 1–related B19 strain was detected in our study, and cross-contamination was excluded by sequence analysis. The low proportion of B19 genotype 1 relative to genotype 3 may indicate that the former was introduced in Ghana from other areas of Africa or from Europe [4].

Three women (with samples M1655, M1964, and M2005) had evidence of recent primary B19 infection, which was characterized by the presence of a relatively high viral load (median, $1.21 \times 10^5$ IU/mL [range, $1.14 \times 10^5$–$3.63 \times 10^5$ IU/mL]), compared with that of the 13 IgM-negative viremic women with persistent infection ($P = .009$). The presence of B19-specific IgM suggested that the infection had occurred during the last trimester of pregnancy [14]. Maternal-fetal transmission was suspected in 2 of these mother-newborn pairs, because of the detection of B19 DNA in the cord blood samples, and it was confirmed in 1 pair (with samples 1964M, 1964C, and 1964B) by the finding of viral DNA in the newborn whole-blood sample (table 1). Cross-contamination from maternal blood was excluded in 1 pair (with samples 2005M and 2005C), because the viral load in the cord blood sample was higher than that in the maternal plasma sample. In addition, sample 2005C contained relatively high titers of IgM, which suggested that there was a fetal immune response. These observations are consistent with reports investigating fetal viremia in the absence of IgM [15]. Unfortunately, no long-term follow-up samples were available to determine the outcomes of these antenatal infections in the newborns.

On the basis of cord blood testing, sample M1655, which had a high viral load and reactive IgM, was from a mother who did not transmit B19. Sample M1655 carried a higher titer of IgG than did those from transmitting mothers (table 1). Such a high titer of presumably neutralizing maternal antibodies might have prevented infection or permitted clearance of B19 in the fetus until there was an undetectable viral load. The concomitant presence of a high viral load and a high titer of presumably neutralizing maternal antibodies might have prevented infection or permitted clearance of B19 in the fetus until there was an undetectable viral load. The concomitant presence of a high viral load and a high titer of B19-specific IgG in the mother might bring into question the neutralizing capacity of these antibodies.

Maternal-fetal transmission most likely occurs through breaks in the placental barrier, which lead to “maternal-fetal transfusion.” Fetal infection depends on the protection provided to the fetus by maternal antibodies, because the immunological immaturity of the fetus probably does not provide it with efficient antiviral defenses even when IgM is produced. Infection may depend on the concurrence of maternal-fetal transfusion.
during the brief time when a high viral load, with or without IgM, is present in the maternal blood.

A low viral load (median, 59 IU/mL [range, 30–5.97 × 10^3 IU/mL]) in the circulation with the concomitant presence of B19-specific IgG suggested B19 DNA persistence in 13 mothers (1.5%), as had been previously observed in other populations [4, 7, 13]. By use of cord blood testing, we found no evidence of intrauterine B19 transmission from 13 persistently infected mothers to their fetuses. The lack of B19 transmission from persistently infected mothers may be related to a viral load below the threshold of infectivity in the presence of various titers of neutralizing antibodies. In addition, the transplacental transmission of B19-specific antibodies may further protect the fetus when it is in contact with B19 DNA from maternal blood. Further studies are needed to determine the minimum infectious dose of B19 in the presence or absence of B19-specific IgG. To our knowledge, no data from systematic studies of vertical transmission from B19 genotype 1 DNA–positive mothers have been reported that could be compared with our B19 genotype 3 data.

In our cross-sectional study, testing for B19 infection was restricted to the time of delivery. Therefore, the occurrence of primary B19 infection early during pregnancy could not be excluded. After acute infection, IgM usually persists for ~3 months but may be detectable for several more months, and the viral load may decrease slowly while the IgG response develops and the clinical symptoms resolve [1, 13]. Adverse pregnancy outcomes were not considered in the present study, and the estimate of the B19 transmission rate may be biased. However, fetal death has been reported to be a rare complication of B19 primary infection [1, 2].

Acknowledgments

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References