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# Risk of fetal hydrops and non-hydropic late intrauterine fetal death after gestational parvovirus B19 infection

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ABSTRACT

*Background:* Risk assessment of parvovirus B19 (B19)-associated fetal complications following gestational B19 infection remains controversial.
*Objectives:* To determine the risk of fetal hydrops or non-hydropic late intrauterine fetal death following acute maternal B19 infection at defined gestational weeks.
*Study design:* Observational cohort study of pregnant women with serologic evidence of acute B19 infection. If available, fetal or neonatal tissue samples from cases complicated by fetal loss or hydrops were investigated for the presence of B19 DNA by polymerase chain reaction (PCR) and/or in situ hybridization (ISH).
*Results:* Of 236 women with known pregnancy outcome, 228 had a live birth and 8 a fetal loss. The observed rate of fetal hydrops for all pregnant women was 4.2% (10/236) (95% confidence interval [CI], 2.1–7.7) and 10.6% (10/94) (95% CI, 5.2–18.7) for those infected between 9 and 20 weeks gestation. Tissue samples from 8 hydrops cases were investigated by PCR or ISH and all were B19 DNA positive. Fetal death occurring during or after gestational week 22 was only observed in one case which was associated with B19-derived fetal hydrops.
*Conclusions:* Our findings demonstrate that although adverse fetal outcome is a rare complication of gestational B19 infection, a relevant risk of fetal hydrops exists particularly for women infected between 9 and 20 weeks' gestation. Cases of B19-derived non-hydropic late intrauterine fetal death were not observed in the present study.



## 1. Background

Human parvovirus B19 (B19) infection during pregnancy can lead to asymptomatic infection of the fetus, spontaneous abortion, fetal anemia, non-immune hydrops fetalis and intrauterine fetal death (IUFD).[1] The frequency of B19 infection in case series of non-immune hydrops is about 5–20% but has been reported to reach 44% in anatomically normal fetuses with moderate to severe ascites.[2,3] In systematic studies on pregnancy outcome after gestational B19 infection, the observed risk of fetal hydrops varied between 0% and 6%.[4–10] Fetuses dying of intrauterine B19 infection before 17 gestational weeks are often not obviously hydropic. Later in gestation, B19-derived IUFD is generally associated with fetal hydrops.[11,12] However two recently published case-control studies from Sweden suggested that B19 is also a common cause of non-hydropic late IUFD.[13,14] The concept of B19-derived non-hydropic late IUFD is not supported by the findings of others.[15]

## 2. Objectives

The present study was undertaken to better define the risk of fetal hydrops or non-hydropic late IUFD following acute maternal B19 infection at defined gestational weeks.

## 3. Study design

In Germany, 30–40% of pregnant women are susceptible to B19.[16] To our experience, history of a contact to a suspected case of erythema infectiosum in the own household, the kindergarten or the elementary school is the most common reason for B19 antibody testing in pregnancy. It is general practice in Germany

*Abbreviations:* B19, human parvovirus B19; CI, confidence interval; EIA, enzyme immuno assay; IgG, immunoglobulin G; IgM, immunoglobulin M; IQR, interquartile range; ISH, in situ hybridization; IUFD, intrauterine fetal death; IUT, intrauterine transfusion; LC, LightCycler; NA, nucleic acid; PCR, polymerase chain reaction; WG, weeks gestation; VP1, viral protein 1; VP2, viral protein 2.

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to request B19 IgG and IgM tests at the time of reported exposure. Susceptible women are usually retested 2–3 weeks later to exclude acute infection. Between October 1999 and September 2002, we investigated blood samples from 15,161 pregnant women for B19-specific antibodies. The present study involved women who had serologically confirmed acute B19 infection. The detection of B19-specific IgM antibodies during initial testing in absence of an IgG seroconversion has to be interpreted with caution. In particular the detection of low titre B19 IgM may be due to late convalescence or false positivity.[17–20] To pinpoint the gestational age at the time of maternal B19 infection most accurately, we considered in the final analysis only women who had a B19 IgG seroconversion or an initially high positive B19 IgM test result. For this purpose, a B19 IgM cut off value was arbitrarily chosen following our previous findings on IgM antibody kinetics in pregnant women with acute B19 infection where B19 IgM index values ≥4.0 had a high predictive value for acute B19 infection within the last 4 weeks.[19] Women whose serum samples were referred from other laboratories or sent for serological analysis following an abnormal ultrasound scan and women with multiple gestation were excluded. The stage of gestation at which B19 infection took place was calculated from the number of completed gestational weeks between the last menstrual period and laboratory confirmed diagnosis of maternal B19 infection. Weekly targeted ultrasound examinations were generally recommended for 10–12 weeks after maternal infection. Information on pregnancy outcome or fetal complications was obtained from the referring gynaecologist/obstetrician or fetal medicine specialist at the end of pregnancy or during invasive prenatal diagnosis (treatment). Fetal death was defined as "death prior to the complete expulsion or extraction from its mother of a product of conception, irrespective of the duration of pregnancy".[21] Stillbirth was defined as fetal death during or after 22 weeks' gestation (WG). If available, fetal, neonatal or placental tissue samples from cases complicated by fetal loss or hydrops were investigated for the presence of B19 DNA by polymerase chain reaction (PCR) or in situ hybridization (ISH). Informed consent was obtained from the parents before post-mortem detection of B19 DNA in fetal tissues.

### 3.1. B19 serology

B19-specific IgM antibodies were identified using a high quality, Food and Drug Administration (FDA)-cleared B19 IgM EIA.[22] According to the manufacturer (Biotrin International), an index value <0.9 or >1.1 indicates sample negativity or positivity, respectively. For the present study we arbitrarily categorized specimens showing a B19 IgM index of ≥4.0 as high positive and specimens showing a B19 IgM index of 1.2–3.9 as low positive. VP2 IgG was determined by a quantitative B19 IgG EIA (Biotrin International) as described elsewhere.[23]

### 3.2. B19 PCR

If available, fetal or neonatal cord blood samples from cases with hydrops were investigated for the presence of B19 DNA by PCR to confirm prenatal B19 infection. Up to September 2001 nucleic acid (NA) was extracted using the manual High Pure Viral NA Kit (Boehringer Mannheim and Roche Diagnostics Mannheim, Germany). The manual extraction was replaced in September 2001 by the MagNA Pure LC automated instrument (Roche Diagnostics Mannheim, Germany) using the MagNA Pure LC Total NA isolation Kit (Roche Diagnostics, Mannheim). Up to March 2000 a VP1 and VP2 endpoint multiplex PCR with visualisation of the PCR-products after gel electrophoresis and ethidium bromide staining was in use.[23] From April 2000 a real-time protocol on the LightCycler instrument (Roche Diagnostics, Mannheim) was established based on a primer/probe system spanning a part of the viral VP1 gene region.[24] Positive controls (B19-positive patients sera with a viral load adjusted to the Parvovirus B19 WHO Standard) and negative controls (sterile water) were included in every test run.

### 3.3. B19 in situ hybridization (ISH)

In situ detection of B19 DNA was performed on dewaxed 5 μm-tissue sections from paraffin-embedded tissues by a protocol described elsewhere.[25,26] The hybridization mixture contained the 35S-labeled RNA antisense B19 probe, which was transcribed in vitro from the 2.5-kb HindIII/Eco RI fragment of cloned B19. After the washing procedures, the tissue slide preparations were autoradiographed and stained with hematoxylin and eosin. Control RNA probes were synthesized from nonrecombinant transcription vector bluescript KS- and from plasmid pCVB3-R1 providing enterovirus-specific RNA probes.[25] As negative controls we used post-mortem tissue samples from fetuses and newborns dying of enterovirus infection. B19 DNA positive bone marrow biopsies were used as positive controls.

## 4. Results

The flow chart of selection, follow-up and outcome of pregnancies is presented in Fig. 1. Acute parvovirus B19 infection was serologically diagnosed in 495 pregnant women during the study period. Of these, 76 had serum samples referred from other laboratories and 156 had low B19 IgM reactivity (median IgM index 2.4, interquartile range (IQR), 1.6–3.2). Of the remaining 263 women, 207 showed high B19 IgM reactivity (median IgM index 5.7, IQR, 4.7–6.5) and 56 had a B19 IgG seroconversion. Median maternal age and median gestational age of the 263 women at time of B19 infection was 33 years (IQR, 31–35 years) and 19 weeks (IQR, 13–28 weeks), respectively. Information on pregnancy outcome was available for 236 eligible women. The risk estimates for fetal hydrops and fetal death following maternal B19 infection at various gestational ages are shown in Table 1. The overall rate of fetal hydrops was 4.2% (10/236) (95% confidence interval [CI], 2.1–7.7). The risk of fetal hydrops was 10.6% (95% CI, 5.2–18.7) in women who acquired B19 infection between 9 and 20 WG. Two cases of non-severe fetal hydrops detected in our study cohort were not investigated for the presence of B19 DNA. Considering only confirmed B19 DNA positive hydrops cases, the observed rate of fetal hydrops for all pregnant women was 3.4% (95% CI, 1.5–6.6) and 8.5% (95% CI, 3.8–16.1) for those infected between 9 and 20 WG. Clinical, pathological and diagnostic details of cases with B19-associated fetal hydrops or fetal death are shown in Table 2. Fetal hydrops resolved spontaneously in one case with generalized skin edema (case no. 2). Two cases (nos. 4 and 7) of fetal hydrops ended-up in fetal death before intrauterine transfusion (IUT). Typical in situ hybridization results from tissue sections of the placenta and different fetal organs are shown in Fig. 2. Whereas in kidney, liver and heart an overwhelming number of B19 infected cells are observed, in tissue sections of the placenta only single B19 positive cells are present. Six fetuses with severe hydrops underwent cordocentesis and were treated by intrauterine transfusion(s). Two (nos. 9 and 14) died during or shortly after the intervention and four (nos. 10–13) survived. Fetal death during or after gestational week 22 was only observed in one pregnancy. The stillborn infant was hydropic and B19 DNA positive. The number of B19-associated hydrops cases was highest during 2001, the year with the highest B19 activity (Fig. 3).