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No detection of human bocavirus in amniotic fluid samples from fetuses with hydrops or isolated effusions

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ABSTRACT

Background: Human bocavirus (HBoV) is a recently identified parvovirus associated with respiratory disease in infants. Animal bocaviruses have been shown to cause intrauterine infection, fetal anasarca and abortion in late gestation.
Objectives: To investigate whether HBoV infection is associated with fetal hydrops, fetal anemia or isolated fetal effusions.
Study design: We determined the prevalence of HBoV and parvovirus B19 (B19) DNA in amniotic fluid samples from fetuses with hydrops, anemia or isolated effusions using different real-time PCR protocols, and the HBoV IgG and IgM positivity rate in pregnant women with fetal hydrops or normal ultrasound findings by a non-commercial virus-like particle-based enzyme immunoassay.
Results: None of 87 amniotic fluid samples tested was HBoV DNA positive. Twelve of 60 fetuses with hydrops or anemia were found B19 DNA positive. Anti-HBoV IgG antibodies were detected in 100% (19/19) and 94% (47/50) of serum samples from pregnant women with fetal hydrops and normal ultrasound findings, respectively. All serum samples were found negative for anti-HBoV IgM.
Conclusion: We suggest that HBoV is not a common cause of fetal hydrops, anemia or isolated effusions. This has to be confirmed by further studies of proven gestational HBoV infection.

1. Background

Until recently, parvovirus B19 (B19) was the only member of the subfamily *Parvovirinae* known to cause disease in humans. Gestational B19 infection is associated with an increased risk of hydrops fetalis, fetal anemia and intrauterine fetal death.[1,2] Close and frequent contact to kindergarten- and primary school-aged children is a significant risk factor for acquiring B19 infection during pregnancy.[3,4] Human bocavirus (HBoV) is a newly identified parvovirus potentially associated with lower respiratory tract infection in children and infants.[5] HBoV was first described by Allander and colleagues in 2005 who detected the virus in pooled respiratory samples.[6] A high virus load in the nasopharynx was associated with viremia. Furthermore, HBoV has been shown to elicit a systemic immune response.[7] The spectrum of HBoV-associated disease in adults has not yet been fully elucidated, especially in pregnant women. Based on results from full length nucleotide sequences as well as nucleotide and deduced amino acid sequences of the two major open reading frames HBoV was grouped together with bovine parvovirus and minute virus of canine (MVC) in the genus *Bocavirus*. MVC was shown to cause intrauterine infection and was associated with fetal myocarditis and fetal anasarca.[9,10]

2. Objectives

To investigate whether HBoV infection is associated with fetal hydrops, fetal anemia or isolated fetal effusions.

3. Study design

3.1. Specimens

In Germany, antenatal ultrasound (level 1) screening is mandatory between 9–12, 19–22 and 29–32 gestational weeks. In the presence of abnormal fetal ultrasound findings pregnant women are generally referred to university or office-based maternal–fetal medicine units for further ultrasound investigations (level 2 or 3) and, if appropriate, invasive prenatal diagnosis to exclude fetal abnormalities such as chromosomal defects, genetic diseases or congenital infections. Furthermore, serological evidence of recent

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Table 1
Information on ultrasound findings, maternal age and gestational age at the time of invasive prenatal diagnosis.

Ultrasound findings	Number of amniotic fluid samples, *N*	Median GA in weeks (IQR) at the time of AF sampling	Median maternal age in years (IQR) at the time of AF sampling
Fetal hydrops, anemia, isolated effusion or localised skin edema[a]	94	20 (17–24)	30 (26–34)
Ventriculomegaly	62	22 (21–25)	30 (26–34)
Polyhydramnion	53	26 (23–30)	29 (27–32)
Other abnormalities (e.g. club foot, lung malformation, organ calcifications)	95	21 (20–23)	31 (26–35)
Normal ultrasound findings	236	16 (15–20)	36 (29–38)
No information available	255	18 (16–21)	31 (26–36)
Total	804	20 (16–23)	32 (27–36)

GA, gestational age; IQR, interquartile range; AF, amniotic fluid.
[a] Fetuses with ultrasonographic signs of brain abnormalities or organ calcifications are not categorized within this group.

maternal infection with TORCH agents may result in invasive prenatal diagnosis, even in the absence of suspicious ultrasound findings.

Between September 2005 and November 2006, the Stuttgart laboratory received a total of 804 amniotic fluid (AF) samples from singleton pregnancies. The samples were referred from university- and office-based maternal–fetal medicine units located throughout Germany to rule out prenatal infections. During initial routine prenatal diagnosis AF samples were investigated for specific TORCH agents using either commercially available PCR assays or thoroughly validated in-house PCR protocols. Information on fetal ultrasound findings, maternal age and gestational age at the time of invasive prenatal diagnosis is given in Table 1. Fetal hydrops, anemia, isolated effusion or localized skin edema was present in 94 cases. PCR results obtained during initial routine diagnosis from the AF samples of the last-mentioned cases are shown in Table 2. Retrospectively, 87 of the 94 AF samples were investigated for the presence of HBoV DNA and B19 DNA at the University of Regensburg (Institute of Medical Microbiology and Hygiene). Among these cases, 19 serum samples were concomitantly collected at the time of invasive prenatal diagnosis. The serum samples were tested for B19-specific antibodies by a commercial B19 EIA (Biotrin International) and for the presence of HBoV-specific antibodies using an in-house HBoV EIA. Furthermore, a convenience sample of serum specimens from 50 pregnant women who had normal ultrasound findings was investigated by the in-house HBoV EIA.

3.2. B19 and HBoV DNA detection (PCR)

During initial routine prenatal diagnosis, B19 DNA was detected in AF samples using a LightCycler (LC)-based real-time PCR as previously described.[11] Immediately after LC-PCR, an additional analytical cycle for temperature melting (Tm) analysis was included. The resulting melting curves were automatically converted by the LC software into melting peaks. Isolates with a Tm value differing significantly from that determined for genotype 1 supposedly belong to genotypes 2 or 3.[12,13]

Table 2
Detection of TORCH agents in 94 amniotic fluid samples obtained from fetuses with hydrops, anemia, isolated effusion or localized skin edema.

TORCH agent	Number of amniotic fluid samples with a positive PCR result/number of amniotic fluid samples tested
Human parvovirus B19	12/94
Human cytomegalovirus	4/73
Toxoplasma gondii	0/53
Human enterovirus	0/14
Herpes simplex virus 1/2	0/7
Varicella zoster virus	0/3

PCR, polymerase chain reaction.

Remaining discarded portions of AF samples from fetuses with hydrops, anemia, isolated effusion or localized skin edema were coded for retrospective (blinded) testing at the Regensburg laboratory. Viral nucleic acid was isolated from 200 μl amniotic fluid and eluted in 100 μl using a QiaAmp DNA blood mini kit (Qiagen, Hilden, Germany). Detection of B19 DNA was performed as described elsewhere.[14] For HBoV, the following primers and probe were used for amplification of HBoV genome sequences by TaqMan real-time PCR: Forward primer 5′-CCA CCT ATC GTC TTG CAC TGC-3′ (nts 2586–2606), reverse primer 5′-TTT TCC CCG ATG TAC TCT CCC-3′ (nts 2619–2639), probe FAM-5′-TCG AAG ACC TCA GAC CAA GTG ATG AAG ACG-3′-TAMRA (nts 2608–2637), positions according to Genbank number DQ000496.1. Duplicate PCR reactions were performed using the TaqMan Universal PCR Master Mix (Applied Biosystems, Weiterstadt, Germany) with 300 nM forward primer, 300 nM reverse primer, 150 nM probe and 200 nM dNTP. An initial denaturation phase of 10 min at 95 °C was followed by 45 cycles at 95 °C (15 s) and 60 °C (1 min). In each run, tenfold serial dilutions of plasmid DNA (HBoV ST2, kindly provided by Dr. Tobias Allander, Karolinska Institute, Stockholm, Sweden)[6] were amplified as positive control and quantification standard. A HBoV DNA negative serum sample was used as a negative control.

3.3. HBoV and B19 serology

Anti-HBoV IgG and IgM antibodies were determined by a HBoV EIA based on virus-like particles (VLPs) containing VP2, expressed from a baculovirus vector.[8] The HBoV EIA was performed as described elsewhere.[15] The cut-off value was determined using serial dilutions of two positive reference sera and the mean absobance of five negative controls. EIA results were divided into different categories according to the degree of absorption as follows: OD < 0.3, negative; OD 0.3–0.4, weakly positive (+), OD 0.5–0.6, positive (++), OD ≥ 0.7, strongly positive (+++). B19 VP2 IgM antibodies were identifyed by a commercially available μ-capture EIA (Biotrin International) as recommended by the manufacturer. VP2 IgG antibodies were determined by use of a sandwich IgG EIA (Biotrin International) as described elsewhere.[16]

3.4. Statistical analysis

Differences in maternal and gestational age at the time of fetal sampling between pregnant women with and without abnormal ultrasound findings were determined using unpaired Wilcoxon test (MedCalc Software).

4. Results

Out of the 804 AF samples, 94 were from pregnancies complicated by (i) fetal hydrops without anemia (*N* = 37), (ii) fetal