Revised Clinical Presentation of Parvovirus B19–Associated Intrauterine Fetal Death

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Adverse pregnancy outcome due to human parvovirus B19 (hereafter referred to as “parvovirus B19”) has been characterized, in numerous reports, as an event that occurs during the first and second trimesters and is strongly associated with symptoms of fetal hydrops. Recent findings have indicated that parvovirus B19–associated intrauterine fetal death (IUFD) is also a problem in late gestation, although its clinical presentation is aberrant, lacking signs of fetal hydrops. We outlined the clinical presentation and assessed the frequency of parvovirus B19 infection in a retrospective analysis of 92 unselected cases of IUFD that occurred during or after gestational week 22. By polymerase chain reaction, parvovirus B19 DNA was detected in 13 (14%) of the 92 cases. Only 2 of the parvovirus B19 DNA–positive cases were hydropic, both representing early IUFDs. This finding indicates that parvovirus B19–associated IUFD in late gestation is a common finding and that hydropic presentation is rare. This knowledge may contribute to a reduction in the number of unexplained cases of IUFD.

Neonatal mortality rates have significantly decreased during recent years because of improved perinatal care, but no comparable reduction in antenatal mortality rates has been observed [1, 2]. This could be explained, in part, by the fact that the etiology of a large proportion of intrauterine fetal deaths (IUFDs) is unknown, and different studies have shown that no identifiable cause can be determined for 12%–50% of stillbirths [3, 4]. Human parvovirus B19 (hereafter known as “parvovirus B19”) was first reported to be associated with IUFD in 1984 [5, 6], and later investigations have given valuable insight into the details of parvovirus B19 infection during pregnancy. The seroprevalence of parvovirus B19 among pregnant women is ~60% [7], which means that 40% of pregnant women are susceptible to primary infection; however, reinfection or reactivation of a latent infection is also possible. The transplacental transmission rate during maternal infection has been estimated to be 33% and 51% in previous studies [8, 9].

Fetal parvovirus B19 infection may be asymptomatic, but it may also result in fetal hydrops and fetal death [10]. The peak incidence of parvovirus B19–associated IUFD has been reported to occur at 23 weeks of gestation [8, 11, 12]. The typical clinical presentation includes fetal loss in the second trimester (with the fetus having a hydropic appearance), as well as maternal parvovirus B19 IgG seroconversion and development of parvovirus B19 IgM antibodies. However, in previous studies, we have presented indications that third-trimester IUFD associated with parvovirus B19 infection is also common, although the clinical presentation differs from that of second-trimester IUFD [13, 14].
cases of third-trimester IUFD, fetal hydrops was a rare finding, and maternal serologic findings did not indicate recent infection, with parvovirus B19 IgG being either absent or present and IgM being absent in all cases studied.

Because most reports are biased by selection for fetal hydrops, in this study, we have sought to clarify the common clinical presentation of parvovirus B19–associated IUFD that occurs during the second half of pregnancy, and we have retrospectively analyzed placental and fetal tissue specimens for the presence of parvovirus B19 DNA by PCR. Specimens were obtained from fetuses for which IUFD occurred during or after gestational week 22, and the specimens were not selected for the presence of fetal hydrops or for infection proven by serological analysis. Obstetric medical records were searched for clinical details regarding the cases.

MATERIALS AND METHODS

Study population and specimen collection. IUFD was defined, according to the recommendations of the World Health Organization, as fetal loss occurring during or after gestational week 22. We evaluated 92 cases of IUFD that occurred in 91 pregnancies (with 1 pregnancy producing twins) that were referred to the Department of Pathology of Huddinge University Hospital (Stockholm, Sweden) from February 1993 through August 1997. The cases were referred from the obstetric departments at either Huddinge University Hospital (n = 73) or Södertälje Hospital (Södertälje, Sweden) (n = 19). According to the official statistical records, a total of 24,531 live births had been referred to the Department of Pathology and were included in the study retrospectively, but 8 cases were later excluded because of a lack of autopsy material, resulting in a total of 92 evaluable cases (68% of the original 135 cases). For each of the 92 cases, paraffin-embedded tissue specimens were available from ≥1 of the following fetal organs: heart, lung, liver, and placenta. In addition, a total of 60 placental specimens were also retrospectively collected (12 from each year of the study) and were used as controls. These control placental specimens had been obtained and sent to the Department of Pathology at the time of delivery as part of the routine clinical procedure for twin pregnancies; the specimens thus were derived from the most normal pregnancies readily available for use as controls. These specimens were processed separately but by use of the same procedure used for the case specimens.

DNA detection. Paraffin-embedded tissue specimens underwent deparaffinization as described elsewhere [14]. DNA was extracted using a commercially available kit (QiaAmp DNA Mini Kit; Qiagen), according to the manufacturer’s instructions, except for a final elution that was performed with 100 μl of distilled water to obtain a more concentrated DNA solution. To minimize the risk of DNA contamination, sterile single-use material was used throughout the process and was discarded between the processing of individual specimens. A total of 2 μl of the DNA solution was used as template in nested PCR for the detection of parvovirus B19 DNA, amplifying a 284-bp fragment of the parvovirus B19 NS1 gene [15]. A “DNA-positive case” was defined by the detection of parvovirus B19 DNA in ≥1 of the available fetal tissue or placental specimens. To detect DNA contamination, water was used as template for every 4 specimens, and 53 known parvovirus B19 DNA–negative placental specimens were randomly inserted among the study specimens and were processed in parallel. No DNA contamination was observed during the process of analysis.

Histopathological examination. Slides of specimens that had been routinely obtained from all cases underwent histopathological reexamination by a pathologist experienced in perinatal pathology, who was unaware of the results of PCR analysis for parvovirus B19 DNA. The tissue specimens were stained with hematoxylin-eosin according to standard procedures and were especially evaluated for the presence of the amphiphilic nuclear inclusion bodies that are considered pathognomonic of parvovirus B19 infection. In addition, immunohistochemical analysis of all tissue specimens available from the 13 parvovirus B19 DNA–positive cases was performed using an antibody that recognized the VP2 protein of the parvovirus B19 capsid (Chemicon International), followed by use of a peroxidase system for visualization.

Collection of clinical data. Obstetric medical records were obtained for all cases, and clinical data were noted. The data were analyzed by a specialist in obstetric medicine who was unaware of the results of PCR analysis for parvovirus B19, and special attention was given to the presence of fetal hydrops, malformations, and signs of infection, as well as the assumed cause of death at the time of IUFD.

Statistical methods. Statistical calculations of differences in parvovirus B19 DNA positivity in the study groups were performed using Fisher’s exact test, and the results were rounded to 2 significant figures.

Ethical approval. The study was approved by the ethical committee of the Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden.

RESULTS

Detection of parvovirus B19 DNA in cases of IUFD. For 13 (14%) of the 92 IUFD cases, PCR detected parvovirus B19 DNA in any of available tissue specimens obtained (table 1);
Table 1. Characteristics of cases of intrauterine fetal death (IUFD) that were defined as parvovirus B19 DNA positive.

<table>
<thead>
<tr>
<th>Fetus</th>
<th>Assumed cause of death at time of IUFD</th>
<th>GA, weeks</th>
<th>Fetal hydrops</th>
<th>Mac</th>
<th>Test results for tissue specimens evaluated for the presence of parvovirus B19</th>
<th>Other histopathological findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Placenta</td>
<td>Heart</td>
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<tr>
<td>1 Unexplained</td>
<td></td>
<td>34</td>
<td>No</td>
<td>No</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>2 Unexplained</td>
<td></td>
<td>41</td>
<td>No</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3 Unexplained</td>
<td></td>
<td>31</td>
<td>No</td>
<td>Yes</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4 Unexplained</td>
<td></td>
<td>33</td>
<td>No</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5 Unexplained</td>
<td></td>
<td>41</td>
<td>No</td>
<td>Yes</td>
<td>+</td>
<td>-</td>
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<tr>
<td>6 Viral Infection (parvovirus B19)</td>
<td></td>
<td>32</td>
<td>No</td>
<td>Yes</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>7 Viral Infection</td>
<td></td>
<td>24</td>
<td>Yes</td>
<td>Yes</td>
<td>+</td>
<td>+</td>
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<tr>
<td>8 Malformation</td>
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<td>32</td>
<td>No</td>
<td>No</td>
<td>NT</td>
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<tr>
<td>9 Malformation</td>
<td></td>
<td>41</td>
<td>No</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>10 IUGR and placental insufficiency</td>
<td></td>
<td>28</td>
<td>Yes</td>
<td>No</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>11 Abruptio</td>
<td></td>
<td>31</td>
<td>Yes</td>
<td>No</td>
<td>+</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>36</td>
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</tr>
</tbody>
</table>

**NOTE.** GA, gestational age; HP, histopathological examination; IS, immunohistochemical staining; IUGR, intrauterine growth retardation; Mac, moderate to severe maceration at autopsy; NT, not tested; PE, preeclampsia; VSD, ventricular septum defect; +, positive; -, negative.
Figure 1. Gestational age of all cases of intrauterine fetal death. The gestational age (in weeks) at the time of IUFD for parvovirus B19 DNA–negative cases (empty bars) and parvovirus B19 DNA–positive cases (filled bars) is shown. *Hydropic parvovirus B19 DNA–positive cases.

this rate of detection was significantly higher than that for control placental specimens, among which no parvovirus B19 DNA–positive case was seen (n = 60) (P = .0017, by Fishers’ exact test). Because the control specimens were exclusively placental, we compared placental specimens from IUFD cases with those from controls. Seventy-six of the 92 IUFD cases had placental tissue specimens available for PCR analysis for parvovirus B19; 6 (8%) of these 76 cases were parvovirus B19 DNA positive, which is a significant difference (P = .0017, by Fishers’ exact test). For 3 of the parvovirus B19 DNA–positive cases (fetuses 3, 5, and 11), parvovirus B19 DNA could be detected in placental specimens only, even though fetal tissues were available for 2 of the cases; therefore, fetal infection could not be verified in these 3 cases. However, the placental specimens obtained from these fetuses did show pathological changes, including varying degrees of edema, chorioamnionitis, infarction, and intervillous hemorrhage. Placental specimens were not available for 3 of the other parvovirus B19 DNA–positive cases of IUFD. In the present study, only placental tissue was obtained for 5 IUFD cases, and only fetal tissue was obtained for IUFD 15 cases. Specimens from all 3 fetal organs and placenta could be obtained for 51 IUFD cases.

Clinical presentation of IUFD cases. Fifteen different diagnoses were found to be the assumed cause of death at the time of IUFD, as determined from a review of the medical records. No diagnosis could be found for 27 (29%) of the 92 cases. Five (19%) of these 27 unexplained cases of IUFD were parvovirus B19 DNA positive. For 2 other parvovirus B19 DNA–positive cases, viral infection was suspected at the time of IUFD (fetuses 6 and 7). The mother of fetus 6 showed serological evidence of parvovirus B19 infection, and PCR of serum samples was positive for parvovirus B19. Pathological investigation of fetus 7 showed pneumonitis with characteristics suggestive of a viral etiology, but the infectious agent was not identified. Malformation was considered the probable cause of death for 2 of the fetuses that were parvovirus B19 DNA positive; 1 of these 2 fetuses (fetus 8) had minor hydrocephalus and a medial cleft palate, and the other fetus (fetus 9) had a ventricular septum defect. A placental complication occurring alone (as in fetus 11) or in combination with either intrauterinegrowth retardation (in fetus 10) or preeclampsia (in fetus 12) was assumed to be the cause of death for 3 fetuses, and maternal diabetes mellitus was thought to be the cause of death for the remaining fetus (fetus 13). For 16 (17%) of all 92 cases, IUFD occurred late in the second trimester (gestational age, 22–27 weeks), and, for the remaining 76 cases (83%), IUFD occurred in the third trimester (gestational age, ≥28 weeks) (figure 1). The mean gestational age at the time of IUFD was 33 weeks (median, 34 weeks; range, 22–42 weeks). For 12 of the 13 parvovirus B19 DNA–positive fetuses, IUFD occurred during gestational week 28 or later, constituting 16% of all third-trimester IUFD cases. Two fetuses were hydropic, with one having IUFD occur in the second trimester (at gestational week 24), and the other having IUFD occur in the early third trimester (at gestational week 28). Three other fetuses (for which IUFD occurred at gestational ages of 33, 34, and 37 weeks) had fetal hydrops but were parvovirus B19 DNA negative.

Seasonal variation of parvovirus B19–associated IUFD. We studied the distribution of parvovirus B19 DNA–negative
and –positive cases over time. To include a longer period of time than that covered by the study reported here, all cases of IUFD included in another study conducted during 1998–1999 were added to our assessment (figure 2) [13]. Clusters of parvovirus B19 DNA–positive cases were seen during each of the following periods: quarter 4 (October through December) of 1993, quarter 2 (April through June) of 1996, and quarter 1 (January through March) of 1998.

**Histopathological evaluation.** Histopathological and immunohistochemical examinations of the parvovirus B19 DNA–positive fetuses were performed (table 1). For 2 parvovirus B19 DNA–positive fetuses, amphiphilic inclusion bodies could be observed, and the results of immunohistochemical staining were also positive. Seven (54%) of the 13 parvovirus B19 DNA–positive fetuses showed moderate to severe maceration on pathological examination.

**DISCUSSION**

We retrospectively investigated the clinical presentation and the frequency of parvovirus B19–associated IUFD. Parvovirus B19 infection was diagnosed by PCR detection of parvovirus B19 DNA in fetal and placental tissues obtained from a total of 92 fetuses evaluated as IUFD cases during 5 consecutive years. Sixty placental specimens obtained from twins delivered at term were included as controls. There was a significant overrepresentation of parvovirus B19 DNA–positive cases among the IUFD cases (14%), compared with the controls.

Even though fetal infection could not be proven in 3 of the parvovirus B19 DNA–positive cases because parvovirus B19 DNA was detected in placental specimens only, it is still possible that parvovirus B19 contributed to the IUFD in these cases. Degeneration of the placenta resulting from inflammation and vasculitis has been reported to be common in cases of parvovirus B19 infection [16, 17], and pathological changes were indeed found in the placental specimens evaluated. Third-trimester IUFD cases were overrepresented in comparison with second-trimester cases in the present study, which may reflect a lower referral rate for earlier cases of fetal deaths. This should not, however, influence the proportional differences in parvovirus B19 PCR positivity noted between second-trimester IUFDs (1 [6%] of 16) and third-trimester IUFDs (12 [16%] of 76) in this study. However, in recent years, efforts to investigate the cause of IUFD have intensified, as have investigations regarding deaths occurring in early gestation; the findings of these investigations have led to the development of detailed postevent protocols, which have contributed to higher rates of referral and positive diagnoses. As a consequence, serological analysis for parvovirus B19 is now part of the routine procedure for assessment of IUFD cases; this was not the case during the study period.

In this study, we conclude that PCR is the most sensitive

**Figure 2.** Distribution of parvovirus B19 DNA–positive and –negative cases of intrauterine fetal death (IUFD) over time. The time of IUFD (quarter [denoted by “Q”] and year [denoted by the last 2 digits of the 4-digit year]) is given for all parvovirus B19 DNA–negative cases (empty bars) and parvovirus B19 DNA–positive cases (filled bars). Cases occurring up to and including the third quarter of 1997 are from the present study, whereas cases occurring during the first quarter of 1998 or later have been reported elsewhere [13].

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method for parvovirus B19 detection, because only 2 (15%) of 13 parvovirus B19 DNA–positive cases could be verified by histopathological and immunohistochemical examinations, which is consistent with our previous findings [13, 14]. Positive findings were observed in spite of the presence of maceration in both cases. It is worth noting, however, that, for one case (fetus 6), the results of histopathological and immunohistochemical examinations of all organs analyzed were positive, whereas PCR was positive for parvovirus B19 in lung tissue only and failed to detect parvovirus B19 DNA in heart and liver tissue. The failure of PCR analysis to detect the DNA could possibly represent false-negative amplification resulting from the high concentration of inhibitory factors in these tissues. An important result in the present study was the successful extraction of viral DNA from paraffin-embedded tissues that had undergone long-term storage. The oldest parvovirus B19 DNA–positive specimen in this study was >8 years old at the time of PCR analysis.

The clustering of parvovirus B19 DNA–positive IUFD cases observed in the present report is consistent with the noted cyclic variation in the prevalence of erythema infectiosum (EI), a childhood disease caused by parvovirus B19 [18, 19]. EI epidemics were reported from Denmark in 1994 and from Scotland in 1998 [20, 21], but, unfortunately, the corresponding epidemiological data were not available from the geographical area covered in the present study. Future studies are needed to establish whether there is any covariation of EI and parvovirus B19–associated IUFD. No absolute causal relationship between parvovirus B19 infection and IUFD was established in this study, because no specific organ manifestations have been demonstrated. The causality must be based, instead, on clinical and laboratory findings and must be established by exclusion of other possible diagnoses. In this context, it is interesting to note that no other explanation could be found for 7 of the parvovirus B19 DNA-positive cases. Five of these cases were unexplained at the time of IUFD and, for 2 other cases, a viral etiology was suspected (parvovirus B19 infection was diagnosed in 1 of them). In these 7 cases, we can find no better suggested cause of death than parvovirus B19 infection. There were 2 cases for which malformations were regarded as the cause of death at the time of IUFD. However, the malformations were most likely too insignificant to cause IUFD, and parvovirus B19 infection remains a more probable cause. A placental complication was diagnosed for 3 other cases and, of course, must be regarded as a contributing cause of death in these cases. However, it is possible that infection with parvovirus B19 aggravated the situation in these cases. Information was scant regarding the case for which maternal diabetes mellitus was diagnosed, and the role played by the parvovirus B19 infection in this case was not clear.

The association of parvovirus B19 infection and fetal hydrops with fetal death in the second trimester is well documented [9]. However, we have found that parvovirus B19 infection in third-trimester cases of IUFD seldom leads to fetal hydrops. It seems as if the frequency of fetal hydrops due to fetal parvovirus B19 infection decreases after approximately gestational week 28, even though we have shown that the frequency of parvovirus B19–associated fetal death does not. The mechanisms involved in the development of fetal hydrops are not fully understood, but severe anemia, along with subsequent congestive heart failure and hypoxia, seem to be important components. Parvovirus B19 specifically targets early erythropoietic progenitor cells and inhibits the production of mature erythroid cells, with anemia as a result. Because the second trimester is a period of very active erythropoiesis, the parvovirus B19–infected fetus probably is particularly prone to develop severe anemia and hydrops during this period [22]. On the other hand, during the third trimester, hematopoiesis is less active, and the erythroid cells begin to contain more adult-type and less fetal-type hemoglobin; as such, the life span of their RBCs is longer, making both the anemia less severe and the development of hydrops less probable.

The maturity of the immune system may also influence the outcome of infection. The fetal immune system is relatively immature during the first half of pregnancy and is not able to produce a significant fetal IgM response. Maternal IgG antibodies probably also contribute to the antiviral response and cross the placenta most readily in the third trimester. Furthermore, infection of hematopoietic progenitor cells residing in the liver during earlier stages of pregnancy may affect the function of the liver itself and thereby contribute to the development of fetal hydrops. In contrast, infection of these cells in later pregnancy, when the hematopoiesis is located in the bone marrow rather than in the liver, is arguably less harmful to liver function. The direct effect of parvovirus B19 infection on the heart is not clear, and, even though no case in our study showed signs thereof, the infection has been associated with fetal myocarditis [23]. However, even without evident myocarditis, arrhythmias and asystole represent possible causes of death in nonhydropic cases.

There are very few reports of IUFD associated with parvovirus B19 in late gestation, and, in our opinion, this may be the result, in part, of the previously reported strong association between parvovirus B19 and fetal hydrops. Most studies reporting the prevalence of parvovirus B19 infection in IUFD have selected hydropic cases and, therefore, are likely to include very few third-trimester parvovirus B19–associated IUFDs. By pointing to the possibility of parvovirus B19 infection in all cases of IUFD, not only in hydropic cases and in cases verified by serological analysis, we hope that the diagnostic routines will be improved and will soon provide more data that would
increase our understanding of parvovirus B19 infection during pregnancy.

Acknowledgments

We thank Angerd Berndtson and Annica Westlund for their skillful assistance and Professor Magnus Westgren for his invaluable advice.

References