Parvovirus B19 Infection in the First Trimester of Pregnancy and Risk of Fetal Loss: A Population-based Case-Control Study

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Because parvovirus B19 infection during pregnancy has been associated with increased risk of fetal loss in small or selected study populations, the authors evaluated the risk in a population-based study. A nested case-control study was conducted by using a population-based screening for syphilis in 3 regions in Denmark from 1992 to 1994. Cases of women with fetal loss were identified in the National Patient Register (n = 2,918), and control women with live-born children were identified in the Medical Birth Register (n = 8,429) by matching on age and sampling week. First-trimester serum samples were tested for parvovirus B19 immunoglobulin M positivity. Parvovirus B19 immunoglobulin M positivity was associated with a 71% increased risk of fetal loss (odds ratio = 1.71, 95% confidence interval: 1.02, 2.86). Adjustment for number of children or stratifying for gestational age at loss did not change the risk estimate. Assuming causality, only 0.1% of fetal losses were attributable to parvovirus B19 positivity, a proportion which could increase to approximately 1% during epidemic periods. In conclusion, acute parvovirus B19 infection during the first trimester of pregnancy was associated with an increased risk of fetal loss. However, the impact on the overall burden of fetal losses appeared small even during epidemics.

Abbreviations: B19, human parvovirus B19; CI, confidence interval; ICD, International Classification of Diseases; IgM, immunoglobulin M; OR, odds ratio; PAR%, population attributable risk percent.

Infection with human parvovirus B19 (B19) primarily takes place during childhood. It is the cause of erythema infectiosum, also known as the “fifth disease.” The infection is contagious, and transmission occurs mainly through respiratory secretions. If a woman is infected during pregnancy, the infection may cause hydrops fetalis or fetal loss through infection of fetal erythroid progenitor cells leading to profound anemia and cardiac failure (1, 2). Cardiac failure may also be associated with myocarditis, which can cause arrhythmias or even cardiac arrest without presence of anemia (3, 4). Currently there is no specific prophylaxis or treatment against B19 infection, but symptomatic treatment of the fetus is sometimes able to prevent a fatal outcome (5, 6).

In high-income countries, fetal loss occurs in 4%-8% of pregnancies that extend beyond the first antenatal care examination at 8–12 weeks’ gestation (7, 8). Preventive strategies are therefore a public concern. In a large Danish cohort of pregnant women, 35% were estimated to be susceptible to B19 infection. Furthermore, the annual rate of seroconversion among susceptible pregnant women during epidemic periods is about 13% (8, 9), and the vertical transmission is about 30% (1, 10). Thus, in a scenario with the extreme assumption that every fetal infection led to fetal loss, then during an epidemic up to 5% of fetal losses could be due to B19 infections in the first trimester during pregnancy ((35% × 30% × 13%/8%) = 4%). However, to assess if there is a need for implementing preventive
strategies, the exact proportion of all fetal losses attributable to B19 infection needs to be estimated in a large, population-based study. In contrast, previous studies used small or selected samples or lacked a control group of noninfected women.

We therefore determined the risk of fetal loss after acute, maternal B19 infection in the first trimester of the pregnancy by taking advantage of national registries and B19-tested serum from a population-based screening of 40,050 pregnant women.

MATERIALS AND METHODS

Study population and data sources

The study was nested in a population of 40,050 pregnant women born in Denmark, who lived in the counties of Copenhagen, Frederiksborg, or Southern Jutland in Denmark. These counties have had a population-based, compulsory screening program for syphilis among pregnant women, which was centralized at Statens Serum Institut in Copenhagen, Denmark. All serum samples drawn during the first trimester (gestational weeks 8–12) for syphilis testing between May 15, 1992, and June 30, 1994, were stored at −20°C until B19 testing took place. A total of 44,302 blood specimens were received during the period, and serum was isolated from 44,283 samples representing 41,511 pregnancies. To avoid problems with dependence between observations, we restricted the material to specimens from singleton pregnancies and to the specimen from the first trimester (gestational weeks 8–12) for syphilis testing. A total of 44,302 specimens were received during the period, and serum was isolated from 44,283 samples representing 41,511 pregnancies. To avoid problems with dependence between observations, we restricted the material to specimens from singleton pregnancies and to the specimen from the first pregnancy if the woman had more than one during the study period, thus leaving 40,050 individual specimens from pregnant women aged 14–49 years (mean age, 28.9 years) as population base for the study.

Cases of women who had experienced fetal loss were identified in the Danish National Hospital Register (11), and potential control women with live-born children were identified in the Medical Birth Register (12) by matching on age (1-year interval) and calendar time when the serum was drawn (1-week interval). This identification of women was possible because both the serum samples and data in national registers are kept under the unique Danish 10-digit identification number assigned to all Danish residents. Furthermore, the Danish National Hospital Register has since 1977 contained information about all nonpsychiatric hospital admissions in Denmark, including diagnoses with fetal loss. Since 1973, the Medical Birth Registry has contained computerized information about birth characteristics, which are mandatorily recorded after delivery, including, for example, vital status (live birth, stillbirth) and age of the woman. To optimize the size of the study population, considering costs for B19 testing, we randomly selected 3 matched controls per case for inclusion in the study. If this was not possible, 1 or 2 controls were selected.

B19 testing

B19 immunoglobulin M (IgM) was measured by an enzyme-linked immunosorbent assay (IDEIA B19 IgM; Dako A/S, Copenhagen, Denmark) in accordance with the manufacturer’s instructions. The absorbance discrimination level was set for each plate of wells as the mean absorbance of 3 of the wells containing a reference serum supplied with the kit. The manufacturer’s definition of IgM positivity in serum in a well on a plate was defined as an absorbance value = a factor 2.0 times higher than the absorbance discrimination level for that plate. The value of the factor is here denoted the “cutoff value.” Serum was successfully IgM tested for 98.8% of the samples.

Fetal loss

Fetal loss is distinguished from later stillbirth by death before or after a specific gestational age that often varies in definition from country to country (e.g., United States: 20 weeks of gestation) (13). Since 2004, the Danish definition of fetal loss has been before 22 weeks of gestation, while fetal deaths later in pregnancy are defined as stillbirths. Fetal death was defined on the basis of the World Health Organization’s recommendation, that is, “death prior to the complete expulsion or extraction from its mother of a product of conception, irrespective of the duration of the pregnancy” (14, p. 514). The gestational age at fetal loss was calculated as the number of weeks between the dates of discharge diagnoses and blood sampling for B19 antibodies, plus 10 weeks because blood for syphilis (and thus B19) testing was drawn at gestational weeks 8–12 when the first antenatal visit to a general practitioner must take place. The range of imprecision for gestational age at fetal loss was thus −2 to +2 weeks because of the antenatal visit interval plus the imprecision of the last menstruation period, as in all previous studies on this subject.

Cases of fetal loss were identified in the Danish National Patient Register by using the discharge diagnoses of the mother. Diagnoses were coded according to the International Classification of Diseases (ICD), Eighth Revision, from 1977 to 1993 and according to the ICD, Tenth Revision, since January 1994. The following codes were used to identify cases of fetal loss: ICD, Eighth Revision, codes 643.0, 643.8, 643.9, 645.1, 645.4, 645.7, or 634.61 and ICD, Tenth Revision, codes O02.1 and O03.0–O03.9. The registration of fetal loss diagnosis in the Danish National Patient Registry has a positive predictive value for hospital files on fetal loss of 97.4% (95% confidence interval (CI): 92.7, 99.5) (15).

Statistical analysis

The possible effect of B19 IgM positivity on the risk of fetal loss was analyzed by conditional logistic regression. Odds ratios with accompanying 95% confidence intervals were used as a measure of relative risk and calculated with and without adjustment for number of children (0, 1, 2, ≥3) and in additional analyses also stratified by gestational age at fetal loss (before and after 12 weeks of gestation). Information on number of children was obtained from the Civil Registration System, which is a continually updated demographic database covering the entire Danish population since April 1, 1968, that includes information on, for example, family relations (16).
Because detection of higher titers of B19 IgM in blood probably increases the likelihood that the tested person is infected (i.e., because the chance of false positive is decreased and test specificity increased), we performed an explorative analysis in which we investigated how the odds ratio of the association between fetal loss and B19 IgM positivity varied by different cutoff levels.

To evaluate the proportion of fetal losses attributable to first trimester B19 infection, we calculated the population attributable risk percent (PAR%), assuming that 35% of all women are susceptible to B19 (8), as PAR% = (((0.35 × p)/4) / (OR − 1))/((0.35 × p)/4)(OR − 1) + 1, where “OR” is our odds ratio estimate of the relative risk of fetal loss associated with B19 IgM positivity, and p is the proportion among susceptible women who become B19 IgM positive during a year. Because the first trimester of pregnancy lasts for a quarter of a year, 0.35 × p must be divided by 4. Maximum likelihood estimation of odds ratios and 95% confidence intervals was performed by using the GENMOD procedure in SAS, version 9.1, software (SAS Institute, Inc., Cary, North Carolina). Two-sided P values were based on likelihood ratio tests, and 95% confidence intervals were based on Wald’s approximation and considered statistically significant if they excluded unity.

Ethical aspects

The study was approved by the Scientific Ethical Committee and the Data Protection Agency of Denmark.

RESULTS

Among the 40,050 pregnant women in the study population, 2,918 cases with fetal loss were traced, and 8,429 matched controls were found. The mean ages of the women in the case and control groups were 30.1 (range: 15.8–46.7) years and 29.9 (range: 14.9–46.3) years, respectively. The proportions of nullipara among cases and controls were 46.6% and 45.5%, respectively.

Table 1 shows the risk of fetal loss according to B19 IgM status during the first trimester of pregnancy. Accordingly, B19 infection during the first trimester of pregnancy was associated with an increased odds ratio of fetal loss (OR = 1.71, 95% CI: 1.02, 2.86). Adjustment for number of children (OR = 1.70, 95% CI: 1.01, 2.84) did not change the odds ratio materially. Stratification for gestational age at fetal loss (before 12 weeks of gestation: OR = 1.31, 95% CI: 0.54, 3.17 and after 12 weeks of gestation: OR = 1.98, 95% CI: 1.05, 3.73) did not show a significant difference (P = 0.45). Using the US definition of fetal loss (<20 weeks of gestation) yielded 2,874 cases and 8,305 controls. B19 IgM positivity was associated with a significantly increased odds ratio of fetal loss (OR = 1.98, 95% CI: 1.05, 3.73) when using a cutoff value of 2.5.

When using a cutoff value of 2.5 or higher to define B19 IgM positivity instead of 2.0 as recommended by the manufacturer, we found that the corresponding odds ratios were stabilized at approximately 2. For instance, when using a cutoff value of 2.5, we found that B19 IgM positivity was associated with a significantly increased odds ratio of fetal loss (OR = 2.33, 95% CI: 1.17, 4.65). When using the US definition of fetal loss (<20 weeks of gestation) and the cutoff value of 2.5 to define B19 IgM positivity, we found that the odds ratio was likewise significantly increased (OR = 2.29, 95% CI: 1.12, 4.68).

Table 2 presents the estimated PAR% of fetal losses for endemic and epidemic occurrence of B19 infections, when assuming a causal association. With an odds ratio of 1.71 for the association between B19 IgM positivity and fetal loss, the PAR% was 0.1% for endemic and 0.8% for epidemic occurrence of B19. When using a cutoff value of 2.5 again to define B19 IgM positivity, we found the PAR% to be increased slightly to 0.2% for endemic and 1.5% for epidemic occurrence of B19.

DISCUSSION

The present population-based study demonstrated that B19 infection during the first trimester of pregnancy was associated with a 71% increased risk of fetal loss. No substantial changes in the relative risk estimate were found for women with a high IgM antibody response to B19 or when

### Table 1. Odds Ratios of Fetal Loss According to Maternal B19 IgM Status During the First Trimester of Pregnancy in a Population-based Case-Control Study Nestled in a Cohort of 40,050 Pregnant Women, Denmark, 1992–1994

<table>
<thead>
<tr>
<th>Maternal B19 IgM Status</th>
<th>Cases</th>
<th>Controls</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>2,894</td>
<td>8,388</td>
<td>1</td>
<td>Referent</td>
</tr>
<tr>
<td>Positive</td>
<td>24</td>
<td>41</td>
<td>1.71</td>
<td>1.02, 2.86</td>
</tr>
</tbody>
</table>

Abbreviations: B19, human parvovirus B19; CI, confidence interval; IgM, immunoglobulin M.

### Table 2. Estimated Proportion of Fetal Losses Attributable to B19 Infection During the First Trimester of Pregnancy (PAR%) by Definitions of Maternal B19 IgM Positivity and by Prevalence of B19 Infection Among Susceptible Women, Denmark, 1992–1994

<table>
<thead>
<tr>
<th>B19 IgM Positivity by IDEIA Test Cutoff</th>
<th>Prevalence of B19 in Pregnant Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endemic (1.5%)a</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td></td>
<td>PAR% 95% CI</td>
</tr>
<tr>
<td>Cutoff 2.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Cutoff 2.5</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Abbreviations: B19, human parvovirus B19; CI, confidence interval; IDEIA, enzyme-linked immunosorbent assay (IDEIA B19 IgM; Dako A/S, Copenhagen, Denmark); IgM, immunoglobulin M; PAR%, population attributable risk percent.

a Endemic periods with 1.5% and epidemic periods with 13% annual incidence of symptomatic B19 infection among pregnant women.
restricting to fetal losses earlier (<20 weeks) in pregnancy. At the population level, our findings are reassuring in that only a very limited proportion of all fetal losses were attributable to B19 infection in the first trimester of pregnancy.

The study had several strengths compared with previous studies. Cases and matched controls were sampled from a large population-based cohort of pregnant women. The use of serologic testing of B19 infection minimized bias due to misclassification; the use of nationwide register data on fetal losses minimized selection bias, and matching was excellent with hospital files in validation studies (15). The matching of cases and controls for exact calendar week of serum sampling strongly reduced any confounding by pathogens potentially co-occurring with parvovirus B19. In addition, the matching for maternal age reduced potential confounding by age-related complications of pregnancies. We also adjusted risk estimates for number of children, because having many children is associated with increased risk of both B19 infection and fetal loss (8, 17). In this context, we considered that risk of fetal loss depends on not only maternal age but also previous fetal losses (17). However, the latter are not a risk factor for B19 and were thus excluded as an adjustment variable. Similarly, other risk factors for fetal loss, such as diabetes, hypertension, and smoking, were not considered confounders because they are not associated with B19 infection. It might be argued that infection in a very small proportion of women could have been misclassified because they were infected shortly prior to pregnancy (false-positive infections) or shortly prior to sampling (false-negative infections), as B19 antibodies persist for 2–3 months and are not detectable until 9–10 days after infection (18). However, because fetal loss is most likely registered correctly regardless of B19 infection, such misclassification would tend to bias our results toward unity and lead to an underestimation of the true effect on risk of fetal loss. Additionally, an underestimation might theoretically occur if gestational age at fetal loss was biased upward (e.g., by imprecision; refer to Materials and Methods), so that some fetal losses at less than 22 weeks were excluded, or if some women with early fetal losses were not registered because the loss did not cause hospitalization. However, such biases are not likely to cause differential misclassification, that is, differing according to B19, and thereby bias the observed excess risk of fetal loss after B19 infection in pregnancy. Acute B19 infection is best detected by using serologic tests, because approximately 50% of infections in pregnant women have been reported to be asymptomatic (19, 20). Withstanding the suspected risk of fetal loss, many public health units today confirm a suspected infection by using several tests, for example, tests for B19 immunoglobulin G, IgM, immunoglobulin G seroconversion, and DNA. However, at the population level, testing for IgM once during pregnancy is more feasible, and the IDEIA B19 IgM test has a reported specificity of 95%–97% (21, 22). A high specificity is especially important when investigating rare exposures, such as B19 infection. We therefore regarded IgM testing sufficient to evaluate the need for national strategies or guidelines in relation to B19 infection and fetal loss.

Whereas several smaller studies reported no association between B19 and fetal loss (20, 23, 24), 2 larger studies found a significant risk increase. One larger study in the United Kingdom of 427 pregnant women with symptomatic B19 infection reported that, during the first 20 weeks of gestation, 15% of the infected women experienced fetal loss compared with 5% in the control group (25). In a similar design, a larger study of 1,018 pregnant women in Germany reported that 11.0% of infected women experienced fetal loss versus 5.4% in the control group (14). Compared with the 2 latter studies, our study results demonstrated a slightly lower excess risk of fetal loss after B19 infection (71% vs. 200%–300%). There are several possible explanations for this difference. One explanation could be that the effect of symptomatic infection is more severe and associated with a larger excess risk of fetal loss than asymptomatic infections. On the other hand, subclinical infections occur more often during pregnancy because of the altered immunologic state associated with pregnancy, and still pathologic effects on the fetus are a recognized concern. Another explanation could relate to the observed time of B19 exposure during pregnancy (weeks 9–16 vs. weeks 8–12 in the present study). For example, gestational weeks 9–16 are physiologically a particularly sensitive time for development of B19-induced hydrops fetalis because of the shortened life span of red blood cells during the hepatic stage of fetal hematopoiesis at this time of pregnancy (14, 25). Finally, the difference may well reflect that our study, in contrast to previous studies, was based on truly population-based material free from selection bias.

The study investigated B19 among pregnancies nearly 2 decades ago. However, B19 epidemics reoccur and tend to follow a 3- to 6-year cycle. To the best of our knowledge, no major changes in this pattern have been reported in recent times. The infection has a substantial subclinical reservoir and, with no available vaccine or medicine that prevents infection, the risk of fetal loss and even infant morbidity remain a public health concern. The present results from a population-based setting do not support major preventive interventions against B19 in the general population (e.g., vaccination or occupational absence for pregnant women in daily contact with young children). Whether specific subgroups (e.g., women with fertility problems) should take special measures against B19 exposure must depend on a case-by-case assessment by professional health personnel.

In conclusion, B19 infection in the first trimester of pregnancy was associated with a 71% increased risk of fetal loss in a population-based setting. However, only a small proportion of all fetal losses may be attributable to B19 infection even during epidemics.

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