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ORIGINAL RESEARCH ARTICLE

Parvovirus B19 DNAemia in pregnant women in relation to perinatal death: A nested case-control study within a large population-based pregnancy cohort

Regine Barlinn[1,2,3] | Lill Trogstad[2] | Halvor Rollag[1,3] | Fredrik Frøen[4,5] | Per Magnus[3,6] | Susanne G. Dudman[1,3]

[1]Department of Microbiology, Oslo University Hospital, Oslo, Norway

[2]Division for Infection Control and Environmental Health, Norwegian Institute of Public Health, Oslo, Norway

[3]University of Oslo, Oslo, Norway

[4]Division for Health Care Services, Norwegian Institute of Public Health, Oslo, Norway

[5]University of Bergen, Bergen, Norway

[6]Center for Fertility and Health, Norwegian Institute of Public Health, Oslo, Norway

Correspondence
Regine Barlinn, Department of Microbiology, Oslo University Hospital, PB 4950 Nydalen, 0424 Oslo, Norway.
Email: reg.bar@ous-hf.no

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Abstract

Introduction: Parvovirus B19 (B19V) is the infectious cause of exanthema infectiosum. In Europe around 40% of pregnant women are susceptible to infection. Having small children at home is the main risk factor for contracting an infection during pregnancy. The association between B19V-infection and perinatal death is not yet settled. The aims of the study were to estimate the association between maternal parvovirus B19 infection in pregnancy and perinatal death, and to assess the significance of a positive B19V PCR in pregnancy.

Material and methods: The study population consists of women included in the Norwegian Mother and Child Cohort Study, a prospective population-based pregnancy cohort of nearly 100 000 women. Blood samples were obtained during weeks 17-18 in pregnancy (M1), at birth, and in umbilical cord blood. Within participants in the pregnancy cohort, 138 cases of perinatal death and 1350 controls with live-born children were included in a nested case-control study. Samples were analyzed with B19V serology and B19V PCR according to a predefined test algorithm. For cases, medical records and laboratory results from hospitals were combined with the results of B19V serology and PCR. The reported causes of perinatal death were categorized using the classification system: Causes Of Death and Associated Conditions (CODAC).

Results: The B19V seroconversion rates were 9.8% for cases and 6.8% for control mothers. The odds ratio for maternal B19V infection in cases compared with controls was 1.28 (95% CI 0.35-4.70), adjusted for age, parity, body mass index and tobacco use. B19V-PCR-positive samples were detected at weeks 17-18 of gestation in both cases and controls. The proportion of positive samples was similar in cases and controls, 24% and 28.2%, respectively. Mothers with PCR-positive M1 samples transmitted B19V vertically in 9.1% of cases and in 11.9% of the controls. Of all perinatal deaths, 53% were attributed to placental pathology or unknown causes.

Abbreviations: B19V, parvovirus B19; C, umbilical cord blood from the child; CODAC, causes of death and associated conditions; M1, blood samples from mothers during in pregnancy week 17-18; M2, blood samples from mothers at birth; MoBa, mother and child cohort study; OR, odds ratio.

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Conclusions: B19V PCR positivity was high and similar in both cases and controls. In our study B19V DNAemia was not seen to be associated with fatal outcome of pregnancy. The clinical significance of B19V DNA detection during pregnancy is uncertain. Caution is needed when diagnosing a B19V infection based only on B19V DNAemia.

KEYWORDS

CODAC, DNAemia, MoBa, parvovirus B19, perinatal death, pregnancy

1 | INTRODUCTION

Parvovirus B19 (B19V) is the infectious cause of exanthema infectiosum, a common disease in childhood.[1] In pregnant women, more than 50% of B19V infections are asymptomatic. B19V is transmitted through respiratory droplets and occurs endemically worldwide with epidemic periods every 3-5 years. Seroconversion rates rise during epidemics. Having small children at home is the main risk factor for infection.[2] In Norway, as in many other countries, around 40% of women of fertile age are susceptible to infection.[3,4] Pregnant women may vertically transmit the virus to the fetus in 1/3 of cases and fetal infection occurs in most cases within 12 weeks after maternal infection.[5,6] Maternal infection during the first 20 weeks of gestation has been associated with fetal death in 9%-11% and fetal hydrops in 3%-4% of pregnancies.[5,7] The association between B19V infection and perinatal death is not yet settled.[8-11] A combination of serological methods and PCR analysis is recommended when investigating a possible B19V infection.[12-14] As low-level viremia may persist after infection, the interpretation of B19V DNA detection in pregnant women is unclear.[15,16] Case-control studies are useful in estimating the association between B19V infections and disease.

Perinatal death rates vary between low- and high-income countries, and the distributions of causes differ. Globally, unexplained stillbirth is the most frequent category, constituting over 30% of all cases. There is a need to understand the consequences of B19V infection later in pregnancy. Virus may or may not cross the placenta barrier, but infection of the placenta trophoblasts without the fetus being infected can still affect fetal development. P-antigen, also called globoside, is the receptor for B19V and is present on the trophoblasts' cell surface. Although not permissive for B19V replication, it can infect and express cytotoxic NS1 proteins, which stimulate apoptosis of the cell and may cause placental insufficiency or infarction.[17,18] A classification system for fetal death is required for comparison of data between studies. In the present study, we have used the Causes Of Death and Associated Conditions (CODAC) system to categorize cases.[19]

The aims of this study were (1) to estimate the odds ratio (OR) for maternal infection with B19V in mothers with and without perinatal death of a child, nested within a population-based pregnancy cohort and (2) to estimate the prevalence and significance of B19V PCR positivity in pregnancy.

Key message

High prevalence of B19V-PCR-positive samples in pregnant women. Caution is needed when diagnosing a B19V infection based only on B19V DNAemia.

2 | MATERIAL AND METHODS

The study population consists of women included in the Norwegian Mother and Child Cohort Study (MoBa)—a prospective population-based pregnancy cohort conducted by the Norwegian Institute of Public Health.[20,21] Participants were recruited from all over Norway from 1999 to 2008. The cohort includes 95 200 mothers, 114 500 children and 75 200 fathers. Blood samples were obtained from mothers during weeks 17-18 in pregnancy (M1) and at birth (M2). Umbilical cord blood from the child (C) was also collected.[22] A total of 1350 controls with available plasma samples and questionnaire data were randomly selected and included (see Supplementary material, Table S1). One control later withdrew from the study and was excluded, leaving 1349 controls eligible for analysis. Of the 1349 controls, 1 control had only the M1 and C samples, and another control had only the M1 and M2 samples. The rest had complete sets of plasma samples.

Within the cohort, 150 women were identified and included as cases from a total of 414 women fulfilling the inclusion criteria. The case definition was death of a fetus after inclusion in the MoBa study independent of gestational age before and during birt, and live born infants who die up to the end of day 6 after birth. Cases with available plasma samples were included. For complete inclusion and exclusion criteria see the Supplementary material (Table S1). During review of the medical records, 1 case was excluded because of incorrect classification, leaving 149 cases eligible for further analysis. Cases were categorized into 2 groups: (1) late miscarriage between week 17 and <22 weeks defined as fetal death and/or expulsion before 22 completed weeks of gestation (n = 11), and (2) perinatal death group as classified and defined in CODAC; born ≥500 g or >22 weeks, stillbirth or death after birth until the end of day 6 (n = 138). In the present study, we focused on the investigation of the perinatal death group, defined above. In this group, the M1 and the M2 biological samples were available from 129 and 62 women, respectively, and 46 C samples were obtained from the children. To maximize the completeness of

data, the medical hospital records for all cases from 32 different hospitals in Norway were reviewed, and relevant data, including laboratory data, were combined with the study results. In addition, medical records were surveyed to find out if analysis of B19V had been performed according to the national guidelines on stillbirth. The timing and the reported causes of perinatal death among the cases were extracted from medical records and categorized according to the CODAC classification system for perinatal death. In addition to designating a primary cause of death,[23] the system also aims to classify associated conditions (AC).[19] The cases and controls were initially tested for B19V IgG and IgM antibodies by an enzyme-linked immunosorbent assay (Institut Virion/Serion Gmbh, Würzburg, Germany). Testing for B19V DNA was performed with primers and probes, targeting the VP1 gene of B19V. Nucleic acid extraction for B19V was performed on MagNA Pure LC (Roche Diagnostics, Basel, Switzerland). Laboratory methods have previously been described in detail.[24] The analytical sensitivity was at least 500 IU/mL as determined by measurements of serial dilutions of the WHO international standard for B19V (NIBSC 12/208). Quantification was performed using 2 quantification standards made from dilutions of the WHO international standard for B19V. High-level viremia was defined as $\geq 10^4$ IU/mL. Low-level viremia was defined as $<10^4$ IU/mL. To validate the results, an alternative B19V PCR assay targeting the VP2 gene was used on a selection of positive and negative study samples at an external test laboratory at the Department of Microbiology, Oslo University Hospital. B19V IgG and IgM antibodies were analyzed according to the test algorithm shown in Figure 1. B19V PCR was performed in M1, M2 and C samples, if B19V IgG had an equivocal result, or if B19V IgM had an equivocal or positive result, and in seroconversion samples. A subset of 150 of the 1349 controls was randomly selected in an additional comparative B19V PCR study with the 129 available M1 samples from the 138 cases. One of the 150 randomly selected controls had no material left for analysis, leaving 149 of the controls eligible for PCR analysis. If available material existed, the corresponding M2 and C samples of the B19V PCR-positive M1 samples were analyzed.

2.1 | Statistical analyses

The sample size was calculated based on the B19V seroprevalence. A sample of 150 cases and 1350 controls was needed to detect an OR for B19V infection of 1.5 with a significance level of .05 and power of 80%. Seronegative women are at risk of B19V infection in pregnancy. The odds of B19V infection for cases and controls were estimated by logistic regression. Odds ratios with accompanying 95% CI were used as the measure of relative risk and were calculated both with and without adjustment for parity, maternal age, body mass index (BMI), and tobacco use in pregnancy. Statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY). The random selection of 150 M1 control samples was done using SPSS.

2.2 | Ethical approval

MoBa has obtained a license from the Norwegian Data Inspectorate (01/4325), and a general approval from The Regional Committee for Medical Research Ethics (S-97045, S-95113). The current study was approved by the Regional Committee for Medical Research Ethics in South-Eastern Norway (2012/374B).

3 | RESULTS

Maternal and pregnancy characteristics for cases and controls are reported in Table 1. Smoking and BMI above 25 kg/m^2 were associated with perinatal death. The controls were more often seropositive (B19V IgG-positive and IgM-negative) (63.1%) than cases (57.4%); the OR adjusted for maternal age and parity was 1.27 (95% CI 0.88-1.83) (Table 2). In 106 of 138 cases, seroconversion was investigated by analyzing available plasma samples or previous laboratory results collected from medical records. Among cases, 4/41 (9.8%) initially seronegative cases seroconverted. Among initially seronegative controls, seroconversion occurred in 33/486 (6.8%). Hence the OR for seroconversion in cases compared with controls was 1.28 (95% CI 0.35-4.70). In the case group, seroconversion occurred in the mother of 1 infant who died 1 day after birth in week 26, 1 infant with antepartum death in week 33, 1 infant with antepartum death in week 36, and 1 infant with intrapartum death in week 40 categorized in the placenta, congenital anomaly, unknown and placenta categories in CODAC, respectively (Table 3).

The B19V PCR results of the M1 samples in cases and the subset of controls that were investigated, showed that B19V-PCR-positive M1 samples were found in 31/129 (24.0%) cases with perinatal death and in 42/149 (28.2%) controls (P = .432). The prevalence of positive B19V DNA in available cord blood samples showed that mothers with PCR-positive M1 samples transmitted B19V vertically to 9.1% of cases and 11.9% of the controls. The distribution of the corresponding M2- and C-sample PCR results, levels of viremia and distribution in seropositive and seronegative samples are shown in Table 4. In both cases and controls, nearly half of the PCR-positive samples were seronegative for B19V IgG and IgM antibodies in the M1 sample. Among the 4 controls with a positive PCR in M2, and a seronegative status in M1, 2 seroconverted and 2 displayed a high level of viremia indicating delayed seroconversion. A complete overview over PCR results and serological status are presented in the Supplementary material (Table S2).

Using the CODAC classification, 86 (62.3%) perinatal deaths were categorized as antepartum, 10 (7.3%) intrapartum, and 41 (29.9%) as early neonatal deaths (live-born children who died within the first week) according to CODAC, of which the majority 22 (53.7%) died within the first 24 hours. One pregnancy (0.7%) was terminated because of severe maternal preeclampsia and resulted in fetal death.

The causal categories “placental” and “unknown” counted 45 (32.6%) and 28 (20.3%) cases, respectively, and constituted 53% of

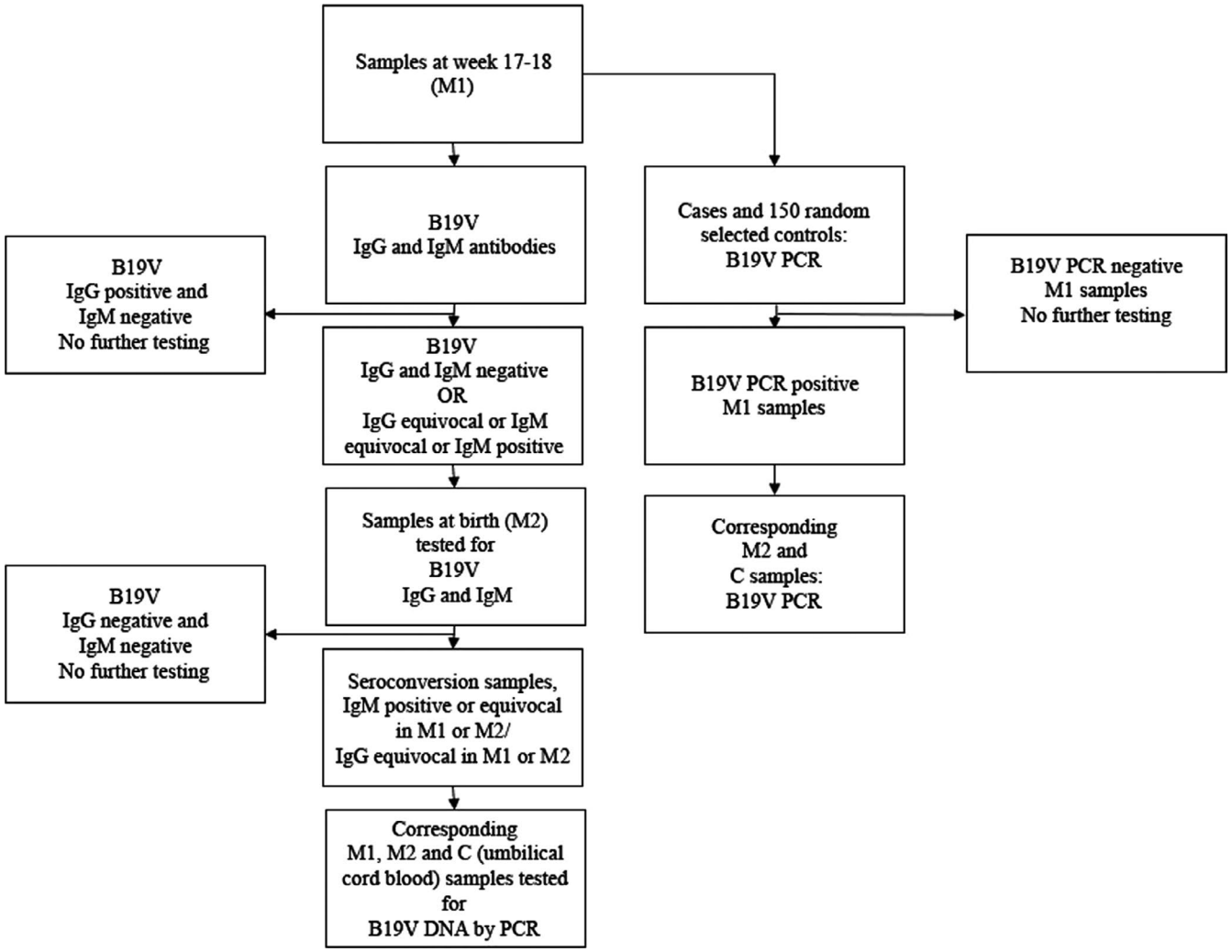

FIGURE 1 Flowchart of the parvovirus B19 test algorithm in a population-based cohort of pregnant women. Plasma was sampled during gestational weeks 17-18 (M1) and at birth (M2) and from the cord blood of newborns (C)

all the perinatal death cases (Table 3). Of these, 18 out of 67 (26.9%) with an available M1 sample had a positive B19V PCR. The "cord" and "unknown" categories had the highest occurrence of a positive PCR with 38.5% and 34.6%, respectively. In 65 (47.1%) of the 138 perinatal death cases, 1 or 2 associated conditions were coded.

According to national guidelines, B19V examination is recommended in all cases of stillbirth. Still, review of medical records showed that only 75 (54.3%) had originally been investigated for B19V (Table 5). None of the women who seroconverted were originally verified in the medical records. Only 4 of the 45 cases with "placenta" as main cause of death, had consecutive samples analyzed for B19V antibodies and 12 women had no test performed. Among the 28 cases with unknown cause of death, 5 had no tests performed, and only 3 had consecutive samples analyzed for B19V antibodies. Viral infection was suspected in 1 of the 149 cases; an antepartum death in gestational week 35, with a massive infarction of the placenta and histologically suspected viral infection; however, no agent was identified in the hospital examination. In our analyses, the M1 sample was missing, but the woman had a B19V IgG- and IgM-positive result in the M2 sample at birth, confirming a recent infection.

4 | DISCUSSION

In the current study, the B19V seroconversion rate is high and it correlates with years of known epidemics in Norway.[24] Both maternal seronegativity against B19V and seroconversion in pregnancy were associated with perinatal death, although the association was not statistically significant. Our study is limited by a relatively small sample of seronegative women for whom we had a maternal plasma sample available after birth. We adjusted for maternal age and parity, which we regarded as the most relevant confounding factors. We also adjusted for maternal prepregnancy BMI and smoking during pregnancy as these factors were associated with perinatal death. Our results add to the literature, but are not sufficient evidence for a clear association between infection with B19V and perinatal death. However, a recent meta-analysis found a significant increase in the risk of fetal loss, spontaneous abortion and stillbirth after maternal B19V infection in.[25]

In this nested case-control study, very high numbers of B19V-DNA-PCR-positive plasma samples were observed at weeks 17-18 of gestation both in women with perinatal death and in randomly drawn controls, corresponding to 24% and 28%, respectively. The prevalence

TABLE 1 Maternal and pregnancy characteristics in 138 perinatal death cases and 1349 population-based controls nested within the Norwegian Mother and Child Cohort Study (MoBa)

Maternal characteristics	Perinatal death cases N = 138	Controls N = 1349	*P*-value
Mean age, years	31.0	30.4	
Nulliparous, n (%)	138 (100)	1349 (100)	
Yes	67	647	.895
No	71	702	
Missing	0	0	
Smoking in pregnancy, n (%)	114 (82.6)	1193 (81.2)	
Yes	17	97	.014
No	97	1096	
Missing	24	156	
Alcohol use in pregnancy, n (%)	116 (84)	1200 (89)	
Yes	16	161	.910
No	100	1039	
Missing	22	149	
Pre-pregnancy body mass index (BMI) Mean (range)	24.9 (17.5-37.0)	23.7 (16.5-44.8)	
BMI >25 kg/m^2, n (%)	137 (99.3)	1324 (98.2)	
Yes	55	409	.027
No	82	915	
Missing	1	25	
Having children <6 y, n (%)	62 (45)	715 (53)	
Yes	54	643	.481
No	8	72	
Missing	76	634	
Having children attending daycare, n (%)	72 (52.2)	761 (56.4)	
Yes	44	497	.476
No	28	264	
Missing	66	588	
Gestational length, weeks, mean (range)	34.5 (22-43)	39.7 (31-44)	

of positive B19V DNA in available cord blood samples showed that mothers with PCR-positive M1 samples transmitted B19V vertically in 9.1% of cases and in 11.9% of the controls. In our study, B19V DNAemia was not seen to be associated with fatal outcome of pregnancy.

Seroprevalence rates in our study are comparable to other studies among pregnant women.[3] It is therefore remarkable that nearly half of both cases and controls with a positive PCR in the M1 sample were also B19V seronegative and did not express serological signs of acute or past infection. For the few samples with a high level of viremia, this finding is most likely the result of an acute infection, but for the majority with a low level of viremia, an acute infection is less likely.

Detectable B19V DNA in tissues and blood without serological evidence of past infection is previously described.[26,27] Interestingly, among the controls, only 2 of the 14 mothers with PCR-positive M2 samples still had a seronegative status in M2. These 2 control women had a high level of viremia, indicating recent infection with delayed seroconversion, or captured antibodies in immune complexes not detectable in serological assays.[14] Antigen persistence after an acute infection should normally provoke an antibody response. However, altered immune response during pregnancy may be a reason for delayed antibody production, which has been described in several case reports.[11,28]

Persistence of B19V at low levels both in immunocompromised and immunocompetent individuals after an acute infection is well documented. In blood donors, positive B19V DNA samples are usually observed in <1% of participants.[15]

Detection of B19V DNA in different tissues has been extensively corroborated, even without serological evidence of recent infection.[29,30] High occurrence of B19V DNA was reported in blood samples from patients with malignancies compared with controls, 50.7% vs 4.5%.[31] A study among kidney transplant patients showed more B19V-PCR-positive cases in the first year after transplantation, correlated with the degree of immunosuppression.[32] Neither of these studies showed a clear association between a positive B19V PCR and clinical symptoms of infection. Hence, another possibility is that altered immune responses or hormonal changes in pregnancy may trigger B19V DNA release from tissues independently of the acute

TABLE 2 Maternal seroprevalence of B19V IgG antibodies at 17-18 wk of gestation, B19V seroconversion and odds ratios with 95% CI for perinatal death in cases compared with controls

Maternal characteristics	Perinatal death cases N = 138	Controls N = 1349	Crude OR (95% CI)	Adjusted[a] OR (95% CI)	Adjusted[b] OR (95% CI)
B19V seroprevalence[c]	129	1318			
B19V antibodies Yes (IgG+, IgM−)	74 57.4%	832 63.1%	Reference	Reference	Reference
B19V antibodies No (IgG−, IgM−)	55 42.6%	486 36.9%	1.27 (0.88-1.84)	1.27 (0.88-1.83)	1.32 (0.88-1.97)
B19V seroconversion[d]	41	486			
B19V seroconversion Yes	4 9.8%	33 6.8%	1.48 (0.50-4.42)	1.50 (0.50-4.52)	1.28 (0.35-4.70)
B19V seroconversion No	37 90.2%	453 93.2%	Reference	Reference	Reference

[a]Adjusted for maternal age and parity.

[b]Adjusted for body mass index, tobacco use in pregnancy, maternal age and parity.

[c]Serology results other than B19V seropositive = (IgG+, IgM−), or seronegative = (IgG−, IgM−) in M1 samples are not taken into calculation (129 cases and 1318 controls).

[d]Only seronegative = (IgG−, IgM−) in the M1 samples are taken into calculation (41 cases and 486 controls).

TABLE 3 Perinatal death cases categorized in the CODAC classification system—cause of death (COD) and associated conditions (AC), number of seroconversion samples and B19V-PCR-positive M1 samples displayed in each category

Causes of death (COD) category	No. of cases (%)	B19V-PCR[a] positive %	B19V Seroconversion, N	COD included AC category[b]	N	B19V-PCR[a] positive %
0 Infection	9 (6.5)	12.5		Infection	14	15.4
1 Neonatal	8 (5.8)	28.6		Neonatal	12	18.2
2 Intrapartum	7 (5.1)	0		Intrapartum	11	20
3 Congenital anomaly	20 (14.5)	20	1	Congenital anomaly	24	20.8
4 Fetal	5 (3.6)	20		Fetal	9	22.2
5 Cord	13 (9.4)	38.5	1	Cord	30	28.6
6 Placenta	45 (32.6)	22	1	Placenta	65	21.7
7 Maternal	2 (1.5)	0		Maternal	29	29.6
8 Unknown	28 (20.3)	34.6	1	Unknown	28	34.6
9 Termination	1 (0.7)	0		Termination	1	0
Total	138 (100)	24	4			

[a]M1 samples for B19V PCR analysis were not available in 9 perinatal death cases, 1 case each in COD categories 0, 1 and 2, and 4 cases in category 6 and 2 cases in category 8.

[b]In 65 of the 138 perinatal death cases, 1 or 2 associated conditions (AC) were coded in addition to COD.

infection, resulting in persistence or merely release of naked non-infectious viral DNA.

A recent study showed that detection of B19V DNA in blood was not a proof of viral replication and that infectious virions were present.[33] They showed that after an acute infection with high viremia, DNA subsequently became degradable by an endonuclease, indicating detection of no longer encapsidated, but naked, DNA. In our study, we found very high numbers of B19V-PCR-positive plasma samples in both cases and controls, which may be due to release of B19V DNA from sites of persistence in tissues.

Detecting B19V DNA through highly sensitive amplification techniques requires careful considerations regarding possibility of contamination. We have taken several measures to avoid, and to detect, possible contamination and we believe that it can be ruled out in the present study.

Moreover, in the current study we demonstrated the value of detecting seroconversion to determine maternal infection, underscoring the importance of having consecutive samples. In Norway, as in several other countries, storage of booking samples in pregnancy is not mandatory, and often not available. Through review of the medical records we proved that although guidelines recommend it, appropriate testing is lacking in most cases of perinatal death.

The classification in CODAC showed that 20% of the perinatal deaths were ascribed to the "unknown" cause of death category. This is a low estimate compared with most other studies.

TABLE 4 Number of M1-B19V-PCR-positive samples among perinatal death cases (N = 31) and controls (N = 42), and the corresponding M2 and C sample PCR results, levels of viremia and distribution in seropositive and seronegative samples

	M1	M1	M2[a]	C[b]
Sample	**B19V-DNA PCR+**	**High-level viremia**	**B19V-DNA PCR+**	**B19V DNA PCR+**
Perinatal death				
Seropositive in M1	17	2	2[c]	1
Seronegative in M1	14	2[d]	0	0
Total B19V PCR+ (%)	31/129 (24%)	4	2/18	1/11 (9.1%)
Controls				
Seropositive in M1	24	2	10[e]	1[f]
Seronegative in M1	18	2	4[g]	4[h]
Total B19V PCR+ (%)	42/149 (28.2%)	4	14/42	5/42 (11.9%)

Note: Seronegative: IgG and IgM negative for B19V antibodies in M1. Seropositive: IgG positive and IgM negative for B19V antibodies in M1. High-level viremia, ≥10^4 IU/mL, low-level viremia, <10^4 IU/mL.

[a]Missing 13 of 31 M2 samples from perinatal death cases.

[b]Missing 20 of 31 C samples from perinatal death cases.

[c]One sample had a high level of viremia, with significant rise in IgG and equivocal IgM result. The corresponding C sample was B19V-DNA-PCR-positive.

[d]Both missing sample in M2.

[e]Three samples with high level of viremia, of which 1 was also IgM positive.

[f]The mother had IgG- and IgM-positive result in M1 and IgG-positive and IgM-equivocal result in M2.

[g]All 4 had a high level of viremia, 2 had a seroconversion and 2 were still seronegative in M2.

[h]Two mothers had a seroconversion and 2 were still seronegative in M2.

TABLE 5 Investigation type in 138 perinatal death cases

				B19V serology performed N (%)		
Type of investigation after adverse outcome	**Placenta N (%)**	**Autopsy N (%)**	**Placenta and autopsy N (%)**	**None**	**One sample analyzed at birth[a]**	**Consecutive samples analyzed[b]**
	99 (71.7)	99 (71.7)	79 (57.3)	63 (46)	62	13

Abbreviations: N, number; placenta, placenta histology report.

[a]Two women exclusively for IgG and 6 women exclusively for IgM.

[b]Of which 5 only had IgG tests performed.

This may be due to a high percentage of autopsy (over 70%) and placental investigations (over 70%) among perinatal death cases. Both autopsy and placental investigation were performed in 57.3% of cases. When including only cases with both autopsy and placenta reports, the percentage with placental pathology as cause of death increased from 33% to 43%. The case with a suspected viral infection, with no causative agent verified at the hospital, demonstrates several points; the value of placenta histology, the difficulty in obtaining the cause of death, and the difficulty in determining whether B19V causes third trimester stillbirth.

A strength of this study is that cases and controls were drawn among nearly 100 000 pregnant women participating in MoBa, a large population-based cohort, over a 10-year time span including both endemic and epidemic B19V periods. Cases and controls were included independently of symptoms. In contrast to studies based on a selection of cases among symptomatic women with acute infection, this study gives valuable insight for a clinical setting. It evaluates the potential effect of screening for B19V infection in perinatal death, and the pitfalls that may arise because the combination of serology and PCR analysis are recommended when investigating B19V infections in pregnancy.[12-14,28,34]

5 | CONCLUSION

The current study has practical implications for diagnosing B19V infection during pregnancy and investigation of the role of B19V as a cause of perinatal death. High prevalence of B19V-PCR-positive samples was detected both in cases and controls. The clinical significance of B19V DNA detection in blood is uncertain and warrants caution in diagnosing a B19V infection during pregnancy or when screening for cause of perinatal death, based on detecting DNAemia only.

Increased knowledge of the immune responses in pregnancy, the impact on serological diagnosis, and development of methods able to differentiate between replicating virus and virus DNA are needed.

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CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

ORCID

Regine Barlinn https://orcid.org/0000-0002-6220-3247

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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