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# Human parvovirus infections during pregnancy:

## Special reference to the child-care employees

**Anita Riipinen**

ACADEMIC DISSERTATION

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# ABSTRACT

Human parvovirus B19 (B19V) is known to cause anemia, hydrops fetalis, miscarriage and intrauterine fetal death (IUFD, i.e., fetal death after 22 completed gestational weeks). Approximately one half of fertile-aged women are B19V-seropositive, and thus immune. Annual seroconversion rates have ranged from 1–13%. Mothers of small children, child-care employees and school teachers are at increased risk of B19V infection.

A recently discovered human parvovirus, human bocavirus (HBoV), has been associated with acute respiratory tract infections, especially in young children. Other animal bocaviruses, bovine parvovirus (BPV) and minute virus of canines (CnMV), are known to cause adverse pregnancy outcomes. The role of HBoV infection in reproduction is unknown.

The aim of this research project was to establish a scientific basis for assessing the work safety of pregnant women and for issuing special maternity leave regulations during B19V epidemics in Finland. The impact of HBoV infection on the pregnant woman and her fetus was also defined. Although it is known that women who are in occupational contact with young children are at increased risk of B19V infection, routine exclusion of B19V-seronegative pregnant employees from work during B19V epidemics is not recommended in most countries. In spite of the many potential risk factors for reproductive health among child-care employees, information on the pregnancy outcome of this occupational group is sparse.

The prevalence of B19V deoxyribonucleic acid (DNA) was studied in women with miscarriages and IUFDs during 7/1992–12/1995 and 1/2003–12/2005. A highly sensitive and specific polymerase chain reaction (PCR) was used. B19V DNA was found in 0.8% of the miscarriages and in 2.4% of the IUFDs. All control fetuses (from induced abortions) were B19V-DNA negative. The findings on hydropic B19V DNA-positive IUFDs with evidence of acute or recent maternal B19V infection are in line with those of two Swedish studies. However, the high prevalence of B19V-related nonhydropic IUFDs noted in the Swedish studies was mostly without evidence of maternal B19V infection and was not found during the third trimester. The data were too small for occupational analysis. Information was also collected on pregnant women with acute B19V infection, and their fetal conditions (N=17) were followed by ultrasound in Helsinki University Central Hospital. Sixteen of these fetuses survived. One third of these B19V-infected mothers worked with children.

HBoV was not associated with miscarriages or IUFDs. Almost all of the studied pregnant women were HboV-IgG positive, and thus most probably immune to HBoV. However, very little is known about the possibility of reinfection, viral persistence or reactivation of HBoV.

All preterm births, perinatal deaths, smallness for gestational age (SGA) and congenital anomalies were recorded among the infants of child-care employees in a nationwide register-

based cohort study. The data contained 13,299 and 12,182 singleton births of the child-care employees and those of the comparison group, respectively, over a period of 14 years. The source population was collected from trade unions and from the register of the National Supervisory Authority for Welfare and Health. Pregnancy outcome, occupation, and employment were determined from national registers. Little or no differences in the results were found between the infants of the child-care employees and those of the comparison group.

B19V seroconversion rates were studied among the pregnant child-care employees (N=320) and those of the comparison group (N=317) during an epidemic in 1992–93 in Finland. The annual B19V seroconversion rate was over two-fold among the child-care employees, compared to the women in the comparison group. The risk increased if the mothers with under three year-old children were excluded (possible child-care leaves indistinguishable from the registers). The child-care employees were more often B19V-seropositive than the women in the comparison group. As anticipated, the seropositivity of the child-care employees increased with age, and years from qualification/joining the trade union. Annual seroconversion rates were 12% among the child-care employees, and 7% among the women in the comparison group.

In general, the personnel of child-care centers are not at increased risk for adverse pregnancy outcome. However, at the population level, the risk of rare events, such as adverse pregnancy outcomes attributed to infections, could not be determined. According to previous studies, seronegative women had a 5–10% excess risk of losing the fetus during the first half of their pregnancy, but thereafter the risk was very low. Therefore, an over two-fold increased risk of B19V infection among child-care employees is considerable, and should be taken into account in the assessment of the occupational safety of pregnant women, especially during the first half of their pregnancy.

# YHTEENVETO

Raskauden aikainen parvorokkovirusinfektio voi johtaa sikiön vesipöhöön (hydrops), keskenmenoon ja sikiökuolemaan. Suomessa ja muualla maailmassa noin puolet lisääntymisikäisistä naisista on parvorokkoviruksen suhteen seropositiivisia ja siten immuuneja. Vuosittain seronegatiivisista naisista 1–13 % sairastaa infektion. Tartuntavaara on lisääntynyt pienten lasten äideillä, leikki-ikäisten lasten parissa työskentelevillä päiväkotityöntekijöillä sekä alakoulunopettajilla.

Uuden parvoviruksiin kuuluvan viruksen, ihmisen bocaviruksen (HBoV), on todettu aiheuttavan hengitystieinfektioita etenkin pienillä lapsilla. Lehmän ja koiran bocavirusten tiedetään aiheuttavan keskenmenoja, mutta HBoV:n vaikutusta raskaudelle ja sikiölle ei tiedetä.

Väitöskirjatutkimuksen tavoitteena oli tuottaa aiempaa perusteellisempaa tietoa parvorokkoviruksen vaikutuksista raskauteen etenkin päiväkotityöntekijöillä, mikä on tarpeen kehitettäessä työsuojelua ja erityisäitiysraha- ja erityisäitiysvapaalainsäädäntöä; lisäksi haluttiin selvittää HBoV:n mahdollista vaikutusta raskauteen. Huolimatta lisääntyneestä altistumisriskistä parvorokkovirukselle työelämässä pienten lasten kanssa työskennellessä ja viruksen aiheuttamista raskausvaikutuksista, selkeitä ohjeita ei ole olemassa raskaana olevien työsuojelun suhteen. Yleensäkin päiväkotityön vaikutusta raskauteen ei ole aiemmin juurikaan tutkittu, vaikka huomattava osa lisääntymisikäisistä naisista työskentelee alalla.

Parvorokkoviruksen DNA:ta löydettiin 0,8 %:lla (1/120) keskenmenneiden ja 2,4 %:lla (4/169) kuolleena syntyneiden (≥22-raskausviikkoiset) sikiöiden kudosnäytteistä ja/tai vastaavista istukoista herkällä ja spesifisellä PCR menetelmällä vuosina 1992–1995 ja 2003–2005. Vertailuryhmässä (raskauden keskeytykset, N=246) ei löytynyt parvorokkovirus DNA-positiivisia kudoksia. Tulokset eivät tukeneet kahdessa aiemmassa ruotsalaistutkimuksessa raskauden loppupuoliskolla löydettyjä korkeita, 14–15 %, parvorokkovirus DNA esiintyvyyksiä kuolleena syntyneillä sikiöillä/vastaavilla istukoilla, usein ilman sikiön vesipöhöä tai serologisesti todettavaa äidin infektiota. Tulokset olivat kuitenkin yhtenevät sikiön vesipöhön ja serologialtaan akuutisti infektoituneiden äitien määrän suhteen. Tutkimusaineisto oli liian pieni äidin ammatillisen riskin selvittämiseen. Lisätarkasteluna tutkimuksessa kerättiin tietoja B19V-infektoituneista äideistä, joiden sikiöiden (N=17) mahdollista infektoitumista seurattiin ultraäänitutkimuksin HUS:ssa. Yhtä lukuunottamatta lapset syntyivät elävinä. Kolmannes infektoituneista äideistä työskenteli lasten parissa.

HBoV DNA:ta ei löytynyt tutkittujen kuolleiden sikiöiden kudoksista tai vastaavista istukoista. Lähes kaikki raskaana olevat naiset olivat HBoV-IgG positiivisia ja siten todennäköisesti immuuneja virukselle. HBoV:n pysyvyydestä kudoksessa, reinfektion tai reaktivaation mahdollisuudesta tiedetään kuitenkin edelleenkin vähän.

Rekisteripohjaisen kohorttitutkimuksen tavoitteena oli selvittää liittyykö päiväkotityöhön lisääntynyt riski lapsen perinataalikuolemalle, ennenaikaisuudelle, matalalle syntymäpainolle, pienipainoisuudelle raskausviikkoihin nähden tai epämuodostumille. Tutkimusaineisto muodostui 13 299 päiväkotityöntekijöiden sekä 12 182 vertailualoilla toimivien naisten yksisikiöisistä synnytyksistä 14 vuoden ajalta. Lähtöaineisto saatiin ammattijärjestöiltä sekä Valviran Terhikki-rekisteristä. Raskaustulokset, ammatti sekä työssäkäynti määritettiin kansallisista rekistereistä. Tutkimustuloksissa ei todettu juurikaan eroja tutkimus- ja vertailuryhmien välillä.

Päiväkotityöhön liittyvää riskiä sairastua parvorokkoon tutkittiin serologisin testein vuosina 1992–1993 raskaana olleilta naisilta Suomessa. Tutkimusaineisto muodostui 320 päiväkotityöntekijöiden sekä 317 vertailualoilla työskentelevien naisten yksisikiöisistä synnytyksistä. Päiväkotityössä riski todettiin yli kaksinkertaiseksi verrattuna vertailuryhmään. Riski lisääntyi, kun alle 3-vuotiaiden lasten äidit suljettiin pois tutkimuksesta mahdollisten hoitovapaiden vuoksi. Lisääntynyttä infektoitumisriskiä kuvaa myös päiväkotityöntekijöillä havaittu suurempi seroprevalenssi, ja sen lisääntyminen suhteessa äidin ikään ja vuosiin valmistumisesta toisin kuin vertailuryhmällä. Epidemia-aikainen vuosittainen infektoituneiden osuus oli 12 % päivähoitotyöntekijöillä ja 7 % vertailuryhmällä.

Päiväkotityöhön, yleisellä tasolla, ei tutkimuksen mukaan liity lisääntynyttä riskiä haitallisiin raskaustuloksiin. Populaatiotasoisella tutkimuksella ei kuitenkaan voida arvioida yksittäisen harvinaisen altisteen, esim. parvorokkoinfektion, vaikutusta raskauteen. Vallitsevan käsityksen mukaan äidin parvorokkovirusinfektioon raskauden ensimmäisellä puoliskolla liittyy 5–10 %:n riski menettää sikiö, kun taas infektio raskauden loppupuoliskolla ei ole vaarallinen sikiölle. Näin ollen parvorokkoviruksen päiväkotityöntekijöille aiheuttama yli kaksinkertainen riski on huomattava ja tulisi huomioida raskaana olevien päiväkotityöntekijöiden työsuojelua kehitettäessä etenkin raskauden ensimmäisellä puoliskolla.

# LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications.

I Riipinen A, Väisänen E, Nuutila M, Sallmén M, Karikoski R, Lindbohm M-L, Hedman K, Taskinen H, Söderlund-Venermo M. Parvovirus B19 infection in fetal deaths. Clin Infect Dis 2008;47:1519–25.

II Riipinen A, Väisänen E, Lahtinen A, Karikoski R, Nuutila M, Surcel H-M, Taskinen H, Hedman K, Söderlund-Venermo M. Absence of human bocavirus from deceased fetuses and their mothers. J Clin Virol 2010;47:186–8.

III Riipinen A, Sallmén M, Taskinen H, Koskinen A, Lindbohm M-L. Pregnancy outcomes among daycare employees in Finland. Scand J Work Environ Health 2010;36:222–30.

IV Riipinen A [*], Sallmén M [*], Meriluoto M, Hedman L, Ojajärvi A, Lindbohm M-L, Surcel H-M, Taskinen H, Nuutila M, Karikoski R, Hedman K, Söderlund-Venermo M. Human parvovirus B19 infection among pregnant child-care employees during an epidemic in Finland. Submitted.

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*equal contribution

The publications are referred to in the text by their roman numerals.

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Helsinki, May 2011

Anita Riipinen

# ABBREVIATIONS

| | |
|---|---|
| A6 | B19V genotype 2 strain |
| AAV | adeno-associated virus |
| Anti-DIG-AP | anti-digoxigenin-alkaline phosphate |
| B19V | human parvovirus B19 |
| BFU-E | erythroid burst-forming units |
| BMI | body mass index |
| bp | base pair |
| BPV | bovine parvovirus |
| BSA | bovine serum albumin |
| CFU-E | erythroid colony forming units |
| CMV | cytomegalovirus |
| CnMV | canine minute virus |
| DIG-11-dUTP | digoxigenin-11-deoxyuridine triphosphate |
| DNA | deoxyribonucleic acid |
| dNTP | deoxyribonucleotide triphosphate |
| EDC | expected date of confinement |
| EIA | enzyme immunoassay |
| ETS | epitope-type specificity |
| FFPE | formalin-fixed paraffin-embedded |
| FMC | the Finnish Maternity Cohort |
| Gb4 | glycosphingolipid globoside |
| HBoV | human bocavirus |
| HIV | human immunodeficiency virus |
| IF | immunofluorescence |
| IgG | immunoglobin G |
| IgM | immunoglobin M |
| IUFD | intrauterine fetal death |
| IUGR | intrauterine growth retardation |
| kb | kilobase |
| LaLi | B19 genotype 2 strain |
| MBR | the Finnish Medical Birth Register |
| MCA–PSV | middle cerebral artery peak systolic velocity |
| MPR | morbilli, parotitis, rubella |
| mRNA | messenger ribonucleic acid |
| NP-1 | nuclear phosphoprotein 1 |
| NS1 | non-structural protein |
| nt | nucleotide |
| OPD | o-phenylenediamine dihydrochloride |
| OR | odds ratio |
| ORF | open reading frame |
| p6 | B19V promoter |
| PARV4 | human parvovirus 4 |
| PCR | polymerase chain reaction |
| qPCR | quantitative polymerase chain reaction |
| RIA | radioimmunoassay |
| RNA | ribonucleic acid |
| RSV | respiratory syncytial virus |
| SES | socioeconomic status |
| SGA | smallness for gestational age |
| ssDNA | single-stranded DNA |
| V9 | B19V genotype 3 strain |
| VP1 | structural viral protein 1 |
| VP2 | structural viral protein 2 |
| VZV | varicella zoster virus |

# 1. INTRODUCTION

*Erythema infectiosum*, also known as fifth disease, and "slapped cheek" disease, is a common childhood exanthematous disease. The clinical symptoms of *Erythema infectiosum* were first described over one hundred years ago. The infection was believed to be a form of rubella, because the symtoms resembled it, and the disease was called rubella-like illness. However, it was soon recognized to be a different disease (Stricker 1899).

A medical virologist, Yvonne E. Cossart discovered human parvovirus B19 in the mid-1970s during the screening of blood for hepatitis B infection by electron microscopy from an asymptomatic patient. Specimen 19 of a panel B contained the unexpected virus, and that is why the virus was named human parvovirus B19 (B19V).

Specific antibodies for B19V were found in children with transient aplastic crisis in the beginning of the 1980s (Pattison et al. 1981). A few years later B19V infection was linked with fifth disease (Anderson et al. 1983), i.e. the fifth of six classic exanthematous childhood diseases to be described. The other exanthematous diseases are measles, scarlet fever, rubella, Duke's disease, and exanthema subitum. Because exanthema subitum occurs typically during infancy, scarlet fever is clinically rare, and measles and rubella have almost disappeared thanks to effective vaccines, the most typical infectious exanthema among pre-school or school-aged children is presently *Erythema infectiosum*.

The first association between B19V infection and adverse pregnancy outcome was reported in 1984 (Brown et al. 1984, Knott et al. 1984), when fetal losses due to intrauterine transmission of the virus from infected mothers were described. B19V can cause fetal anemia, which may lead to cardiac insuffiency, hydrops fetalis and finally even fetal death. Also, B19V can directly infect cardiac cells, causing carditis. The greatest risk for fetal loss due to B19V was during the first half of pregnancy, when the fetus is most vulnerable to hematological changes and the fetal immune system is immature. In the course of the years, B19V has also been linked with artropathy/arthritis especially in adults, and with neurologic diseases, hepatitis, and various other diseases.

A new human parvovirus, human bocavirus (HBoV), was first described in clinical specimens from children with respiratory tract infections in 2005 (Allander et al. 2005). HBoV has been associated with acute respiratory infection usually among children under two years of age. HBoV is closely related to bovine parvovirus (BPV) and minute virus of canine (CnMV), which are classified as belonging to the *Bocavirus* (i.e. bovine/canine). Animal bocaviruses have been associated with respiratory tract infections and gastroenteritis, like HBoV, but also with adverse pregnancy outcomes. The effect of HBoV on pregnancy and the fetus is still unknown.

Although B19V infection is known to cause adverse pregnancy outcomes, in most countries routine exclusion of B19V-seronegative pregnant employees from a workplace with a B19V outbreak is not recommended. The present study investigates the incidence and fetal effects of B19V infection, with special reference to employees in child-care centers. The occurrence of HBoV DNA in miscarriages and IUFDs was also studied. The initial starting points for this study were the necessity to establish a scientific basis for assessing the occupational safety of pregnant women, and to formulate special maternity leave regulations during B19V epidemics.

# 2. REVIEW OF LITERATURE

## 2.1. Taxonomy of parvoviruses

Parvoviruses were discovered in the 1960's, and today they are known to be common, and host-specific animal pathogens, and to cause systemic infections. Parvoviruses are nonenveloped, single-stranded, small DNA-containing viruses. The name parvum means small in Latin. Transplacental parvovirus infections have been described for several animal parvoviruses. The *Parvoviridae* family is divided into two sub-groups (Table 1): *Parvovirinae* infecting vertebrate cells, and *Densovirinae* infecting invertebrate cells. *Parvovirinae* is further subdivided into five genera: 1) *Parvovirus*, 2) *Dependovirus*, 3) *Erythrovirus*, 4) *Bocavirus*, and 5) *Amdovirus*. The sixth genus, *Partetravirus*, has been proposed. Members of the genus *Parvovirus* can replicate autonomously in actively dividing cells; most of the *Dependovirus* members need a helper virus to replicate; those of *Erythrovirus* need erythroid cells to replicate. Only members of the *Dependovirus*, *Erythrovirus* and *Bocavirus* are known to infect humans.

Table 1. Taxonomy of parvoviruses.

| Family | Subfamily | Genus | Viruses (Type species) |
|---|---|---|---|
| *Parvoviridae* | *Parvovirinae* (vertebrates) | *Parvovirus* | *Minute virus of mice* |
| | | *Dependovirus* | *Adeno-associated virus* |
| | | *Erythrovirus* | *Human parvovirus B19* |
| | | *Bocavirus* | *Bovine parvovirus* |
| | | *Amdovirus* | *Aleutian mink disease virus* |
| | | *Partetravirus** | *Human partetravirus** |
| | *Densovirinae* (invertebrates) | *Densovirus* | *Junonia coenia densovirus* |
| | | *Iteravirus* | *Bombyx mori densovirus* |
| | | *Brevidensovirus* | *Aedes aegypti densovirus* |
| | | *Pefudensovirus* | *Periplanta fuliginosa densovirus* |

*new name proposed

## 2.2. Parvovirus B19

### 2.2.1. Morphology

Human parvovirus B19 (B19V) is a small, nonenveloped virus with a single-stranded DNA genome of 5.6 kb, and with a diameter of 20–25 nm. Each virus capsid consists of 60 proteins in an icosahedral structure. The two genomic ends contain identical inverted terminal repeats of 380 nucleotides that are imperfect palindromes and form hairpin loops (Shade et al. 1986, Astell & Blundell et al. 1989, Deiss et al. 1990; Figure 1). These loops consist mainly of GC-pairs. The genome of B19V contains only one functional promoter, p6, which is located in the left end of the viral genome. The virus has no lipid envelope, which makes it extremely resistant to most of the antiviral procedures such as solvent and heat treatments. B19V can be inactivated by formalin, β-propiolactone, gamma irradiation and oxidizing agents (Heegaard et al. 2002b). B19V-contaminated blood products are inadvisable as medical therapies for patients.

Approximately 70% of the mass of the B19V is protein and remainder is DNA. B19V encodes three major viral proteins (Figure 1) – two structural capsid proteins VP1 (83 kDa) and VP2 (53 kDa), one non-structural protein NS1 (77 kDa) (Ozawa et al. 1987) – and two minor proteins (11 kDa and 7.5 kDa).

Two structural proteins

The major structural protein, VP2, accounts for 96% of the total capsid proteins. The ratio between VP1 and VP2 is thought to be due to the relative inefficiency of VP1 in mRNA translation. VP1 and VP2 are encoded by overlapping reading frames. VP1 differs from VP2 by an additional 227 amino acids at the amino terminus. The genes for structural capsid proteins are located in the right end of the genome. VP2 mediates receptor binding. Both structural proteins contain epitopes for neutralizing antibodies (Sato et al. 1991). Recombinant VP1 and VP2 can be expressed in bacterial, mammalian and insect cells.

Non-structural protein

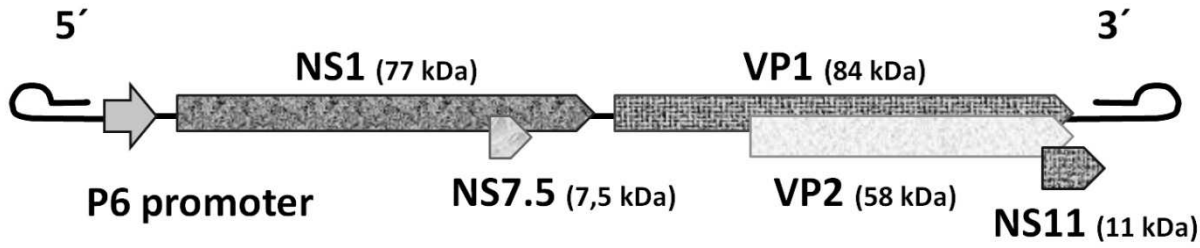
The non-structural protein of B19V, NS1, consists of 671 aminoacids, and has a molecular mass of 77 kDa. The gene for NS1 is located in the left-hand side of the genome. NS1 is known to be involved in viral replication (Ozawa et al. 1986), activation of virus and cellular gene transcription (Doerig et al. 1990, Sol et al. 1993, Moffatt et al. 1996). NS1 induces cell death by apoptosis by the pathway that involves caspase 3, and is inhibited by Bcl-2 (Moffat et al. 1998). NS1 may also play a role in the pathogenesis of autoimmune diseases. In persistent or prolonged infections the prevalence of NS1-specific antibodies may be as high as 80% and therefore NS1 may be an indicator of chronic or more severe courses of B19V infection (von Poblotzki et al. 1995). However, all studies have not supported this conclusion (Jones et al. 1999). Elevated NS1-specific immune reactions have been observed in pregnant women infected with B19V (Hemauer et al. 2000), and that has been supposed to enhance the

risk for fetal infection and adverse pregnancy outcome. However, Searle et al. (1998b) did not find an association between the development of anti-NS1 antibodies and the occurrence of fetal complications.

Small proteins

The genes for the small structural proteins (7.5 kDa and 11 kDa) are located in the centre and at the right end of the viral genome. The role of these proteins in virus pathogenesis is still unknown.

Figure 1. Structure of the B19V genome.




## 2.2.2. Pathogenesis and infection

The B19V life cycle includes attachment of the virus to the host cell receptors, penetration (endocytosis), uncoating, DNA replication, ribonucleic acid (RNA) transcription, protein translation, assembly of virions, and finally cell lysis with release of the virions (Heegaard et al. 2002b). The major cellular receptor for B19V is P antigen (Brown et al. 1993), which is a neutral glycosphingolipid globoside (Gb4). There exist two common globoside antigens P1 and P2, and one less common antigen Pk. P antigens occur on the surfaces of erythrocyte progenitor cells, cardial, synovium, endothelial, placental trophoblast and fetal hepatic and myocardial cells (Brown et al. 1993, Cooling et al. 1995). Individuals, who lack P antigen (1:100 000), are resistant to B19V infection (Brown et al. 1994b).

Because B19V can code for only a few proteins and have no polymerase of its own, it is dependent on actively dividing cells for replication. Like other parvoviruses, B19V replicates in the host cell nucleus. The cell has to go through S phase in order for B19V to replicate. Fetuses have a vast number of cells in active mitosis making them particularly vulnerable. B19V infects and replicates mostly in the erythroid precursors CFU-E (erythroid colony forming units) and BFU-E (erythroid burst-forming units) (Ozawa et al. 1986, Srivastava et al. 1988, Brown et al. 1995). In addition to cytolytic activity, B19V carries also an apoptosis-inducing factor, and an ability to induce cell cycle arrests at either G1 or G2 phases, which can result in massive destruction of erythroid progenitor cells leading to anemia (Chisaka et al. 2003). Lymphocyte, granulocyte and platelet counts may fall during B19V infection.

Reactivation of persistent, latent parvovirus infection during pregnancy has been detected in animals (Siegl 1984), but is very rare in humans. Reinfection of B19V in a pregnant woman and in immunocompromised subjects following renal transplantation has, however, been described (Pillay et al. 1991, Cassinotti et al. 1994).

### 2.2.3. Genotypes

The genome of B19V was first cloned in 1984 (Cotmore et al. 1984). Presently, B19V is divided into three genotypes: genotype 1 (prototype), 2 (A6- and LaLi-like), and 3 (V9-like) (Nguyen et al. 1998, Hokynar et al. 2002, Nguyen et al. 2002, Servant et al. 2002). These three genotypes differ by approximately 10% at the nucleoside level. The most striking variation is observed within the promoter area, in which the three genotypes differ by >20%. Phylogenetic analysis have revealed two subgroups within genotypes 1 and 3 (Toan et al. 2006, Parsyan et al. 2007). No disease-specific genotypes have been described.

Genotype 1 is predominant consisting over 90% of the studied population (Hübschen et al. 2009), whereas genotypes 2 and 3 are rare among symptomatic European patients. However, genotype 2 has been found at a higher frequency in tissues of persons born before 1973, leading to the hypothesis that genotype 2 has almost disappeared from circulation (Norja et al. 2006). Other studies have supported this hypothesis (Manning et al. 2007, Kühl et al. 2008, Schenk et al. 2009). Genotype 3 has been detected endemically in Ghana and in Brazil (Candotti et al. 2004, Sanabani et al. 2006, Keller et al. 2009), and sporadically in France, in the United Kingdom and in the United States (Ngyuen et al. 1998 and 1999, Servant et al. 2002, Cohen et al. 2006, Rinckel et al. 2009). In Finland, among 140,160 blood-donor units, none of the B19V DNA-positive units contained detectable levels of B19V genotypes 2 or 3, but all presented with genotype 1 (Hokynar et al. 2004). However, genotype 2 DNA occurred in the skin of 47% of healthy B19V-seropositive adults (Hokynar et al. 2002).

### 2.2.4. Tissue persistence

After acute infection, residual viral DNA of all B19V genotypes can persist in tissues for decades or even lifelong (Söderlund et al. 1997, Norja et al. 2006, Corcioli et al. 2008). B19V DNA has been shown to persist in bone marrow (Sasaki et al. 1995, Cassinotti et al. 1997 and 1998, Lundqvist et al. 1999, Heegaard et al. 2002a, Manning et al. 2007), synovial tissues (Saal et al. 1992, Söderlund et al. 1997, Cassinotti et al. 1998, Hokynar et al. 2000), myocardium (Kühl et al. 2003, Küethe et al. 2009, Schenk et al. 2009), liver (Eis-Hübinger et al. 2001, Wong et al. 2003, Abe et al. 2007, Wang et al. 2009), tonsils (Norja et al. 2006), skin (Hokynar et al. 2002, Vuorinen et al. 2002), testis (Diss et al. 1999), muscle (Chevrel et al. 2000), lymphoid tissues (Manning et al. 2007), and brain (Hobbs et al. 2006, Manning et al. 2007, Grant et al. 2009). It has been reported that some individuals could be infected with

two genotypes, and persistence of two B19V genotypes can probably occur in the same tissue (Schneider et al. 2008).

### 2.2.5. Epidemiology

B19V is a global and common infectious pathogen in humans. B19V infection appears all through the year, but the highest season is in late winter and spring, with major epidemics every few years (Crowcroft et al. 1999, Enders et al. 2007). In Finland, the documented major B19V epidemics have occurred in 1993, and in 2010. A B19V epidemic has also been reported in 1993 and/or 1994 in England & Wales (Smoleniec et al. 1994b, Miller et al. 1998), in Sweden (Skjöldebrand-Sparre et al. 2000), and in Denmark (Jensen et al. 2000). There is also evidence of an epidemic during 1997 and/or 1998 in Germany, Netherlands, and Sweden (Skjöldebrand-Sparre et al. 2000, van Gessel et al. 2006, Enders et al. 2007). However, Tolfvenstam et al. (2001) reported no epidemic in Stockholm area in 1998.

B19V is most commonly transmitted by respiratory droplets, but also by blood products (Anderson et al. 1985, Lyon et al. 1989, Heegaard et al. 2000) or trans-placentally during pregnancy. Contaminated environmental surfaces have been identified as one potential source for transmission of B19V (Dowell et al. 1995). Transmission has also been described among laboratory staff handling B19V (Cohen et al. 1988b). Fecal-oral transmission has not been documented.

B19V infection is most typical among children 3–10 years of age (Enders et al. 2007, Mossong et al. 2008). In the early postnatal period (0–3 months) <1% of infants have IgM antibodies, but >60% have maternally derived B19V IgG antibodies, which drop to ≤20% around the age of one year (Eis-Hübinger et al. 1998, Miller et al. 1998, Enders et al. 2007). Seropositivity increases gradually being 2–35% among children one to five years, 15–60% among children six to 19 years, 30–80% in adults, and even more than 80% in the elderly population (>70 years) (Andersson et al. 1986, Cohen et al. 1988a, Koch et al. 1989, Skjöldebrand-Sparre et al. 1996, Eis-Hübinger et al. 1998, Heegaard et al. 2002b, Röhrer et al. 2008). In Europe, seroprevalences of B19V have been 35–81%, being the lowest in Spain and the highest in Sweden (Cohen et al. 1988a, Gratacós et al. 1995, Skjöldebrand-Sparre et al. 1996, Valeur-Jensen et al. 1999, Jensen et al. 2000, Alanen et al. 2005, van Gessel et al. 2006, Enders et al. 2007, Mossong et al. 2008). The corresponding proportions are 38–46% in Asia (Yaegashi et al. 1999, Ooi et al. 2002), 64% in Australia (Karunajeewa et al. 2001), 52–58% in Africa (Schwarz et al. 1989b), 60% in North America (Adler et al. 1993), and 43% in South America (de Freitas et al. 1990). In some isolated populations – Brazilian tribes and Rodriguez Island – the seroprevalence of B19V is low, and almost all people are susceptible to B19V (Schwarz et al. 1989b, de Freitas et al. 1990). Differencies between these studies could depend on geographical situations, but also on laboratory methods.

Among blood donors the occurrence of B19V viremia has ranged from 0.01% to 0.6% (O'Neill et al. 1992, McOmish et al. 1993, Yoto et al. 1995, Jordan et al. 1998, Zaaijer et al. 2004, Schmidt et al. 2007, Matsukura et al. 2008).

Major risk factors for exposure to B19V are contact with small children (Valeur-Jensen et al. 1999, Enders et al. 2007, Röhrer et al. 2008), and the number of children at home (Röhrer et al. 2008, van Rijckevorsel et al. 2009). Women of low socioeconomic status and those professionally involved with children are more likely to be B19V-seropositive (Gilbert el al. 2005, van Gessel et al. 2006). The risk of B19V infection among susceptible adults following household exposure to a B19V-infected person is approximately 50%, and following school exposures during outbreaks, 20% to 30% (Anderson et al. 1990, Gilbert et al. 2000). B19V infection is supposed to affect males and females in equal numbers, but in some studies females were more often B19V-seropositive than males (Koch et al. 1989, Lin et al. 1999, Röhrer et al. 2008). The seroprevalence of B19V in urban townships has been higher than that in rural townships (Lin et al. 1999, Röhrer et al. 2008).

## 2.2.6. Clinical manifestations

### 2.2.6.1. Prodromal features

In a voluntary infection study, B19V viremia peaked 8–9 days after inoculation, and lasted 3–7 days (Anderson et al. 1985). The flu-like prodromal features – malaise, headache, sore throat, coryza and low-grade fever – coinsided with the time of viremia.

### 2.2.6.2. Asymptomatic infection

Subclinical B19V infection is a common finding in both adults and children. Among adults, one third (Harger et al. 1998), and among children one fifth (Gillespie et al. 1990) has been reported to be asymptomatic. Because serum DNA levels seem to be similar between asymptomatic and symptomatic patients (Enders al. 2006), viremia has probably no correlation with the clinical picture.

### 2.2.6.3. Erythema infectiosum (fifth disease)

*Erythema infectiosum* is a common childhood rash disease. Rash is a typical symptom in B19V-infected children, but also 40–50% of adults may have this symptom (Reid et al. 1985, Woolf et al. 1989, Harger et al. 1998). A maculopapular rash appears usually by days 16–18 (Anderson et al. 1985, Bell et al. 1989), when the person is no longer infectious. The rash begins on the face (slapped cheeks) and spreads to the neck, trunk, buttocks and extremities. The rash can be pruritic, and is more intense after exposure to sun and exercise (Bell et al. 1989). The rash usually coincides with the appearance of B19V IgG antibodies, suggesting that it is immune mediated; local depositions of immune complexes result probably in rash.

### 2.2.6.4. Arthropathy

Several viral agents, such as rubella virus, Epstein-Barr virus, varizella zoster virus (VZV), cytomegalovirus (CMV), hepatitis B and C viruses have been associated with arthropathy. Of the B19V-infected children arthropathy occurs in 10–20%, while it is the most typical symptom in adults affecting 45–80% of them (Anderson et al. 1984, Woolf et al. 1989, Kerr et al. 1994, Harger et al. 1998), especially females (Reid et al. 1985, White et al. 1985). The most common manifestation is symmetrial peripheral polyarthropathy affecting the wrists, fingers, ankles and knees (Reid et al. 1985, Woolf et al. 1989, Harger et al. 1998). Like the rash, the arthropathy has been suggested to be immune mediated, and it usually appears simultaneously with B19V-IgG antibodies, when the person is no longer infectious. Arthropathy usually resolves within two weeks (Reid et al. 1985, Bell et al. 1989, Woolf et al. 1989), but in 20% of patients it may last over two months, or even years (Dÿkmans et al. 1986, Woolf et al. 1989).

B19V has been suggested to be an ethiological factor of rheumatoid arthritis in some (Saal et al. 1992, Takahashi et al. 1998, Munakata et al. 2005, Chen et al. 2006), but not in all studies (Lefrere et al. 1985, Kerr et al. 1995, Söderlund et al. 1997). The rheumatoid arthritis is often progressive and may lead to destructive change of the affected joint. However, B19V arthritis seems not to cause permanent damage to bones or joints (Jawad et al. 1993).

### 2.2.6.5. Hematologic disorders

Immunological and viremic diseases, drugs and myelodysplasia can cause pure red cell aplasia. B19V is one of the infectious causes of anemia affecting the final stage of the red cell maturation leading to both hemolysis and red cell aplasia. In B19V infection the aplastic stage lasts about 7–10 days. Reticulocytopenia appears from one week up to three weeks after B19V inoculation, followed by a drop in hemoglobin concentration (Anderson et al. 1985). Because the life span of a red blood cell is normally 120 days, hemoglobin levels of healthy persons usually stay stable. For patients with chronic hemolytic anemia – sickle-cell anemia, hereditary spherocytosis, β-thalassemia, or chronic autoimmune hemolytic anemia – with a rapid erythrocyte turnover, of approximately 5–15 days, B19V infection may cause life-threatening red cell aplasia (Pattison et al. 1981, Serjeant et al. 1981, Anderson et al. 1982 and 1986, Chorba et al. 1986, Serjeant et al. 2001). In immunosuppressed patients B19V infection can lead to chronic bone marrow suppression and chronic anemia (Kurtzman et al. 1988).

Lymphopenia, neutropenia and thrombocytopenia can be observed one week after B19V infection (Anderson et al. 1985). A few studies have associated B19V with fetal trombocytopenia (Forestier et al. 1999, de Haan et al. 2008), and with idiopathic thrombocytopenic purpura (Wright et al. 1991, Murray et al. 1994).

### 2.2.6.6. Myocarditis and cardiomyopathy

Myocarditis and cardiomyopathy can be caused by toxins, drugs, and systemic or infectious diseases (Feldman et al. 2000). Enteroviruses have been reported to be the most common cause of myocarditis and dilated cardiomyopathy (Baboonian et al. 1997, Feldman et al. 2000). At present, in a growing number of studies, B19V DNA has been found in the hearts of patients with myocarditis, cardiomyopathy, and idiopathic left ventricular dysfunction (Schowengerdt et al. 1997, Pankuweit et al. 2003, Bültmann et al. 2005, Tschöpe et al. 2005, Kühl et al. 2005 and 2008, Mahrholdt et al. 2006, Bock et al. 2010). Unfortunately, in all studies control groups were not involved. Also, in these studies serology was not performed, and thus acute B19V infection could not be confirmed or ruled out. However, in some case studies the patients with myocarditis have had serologically confirmed acute B19V infection (Papadogiannakis et al. 2002, Dina et al. 2008).

### 2.2.6.7. Hepatitis

Fulminant hepatitis can be caused by hepatotropic agents, metabolic, toxic, immunological, and infectious causes. Acute B19V infection has been diagnosed, and/or B19V DNA has been found in serum of patients with acute, otherwise unexplained hepatitis (Yoto et al. 1996, Hillingso et al. 1998, Sokal et al. 1998, Abe et al. 2007), but in some studies B19V infection is supposed to be only an innocent bystander (Wong et al. 2003). B19V infection is also suggested to cause acute fulminant liver failure requiring liver transplantation (Langnas et al. 1995, Karetnyi et al. 1999).

### 2.2.6.8. Neurologic disorders

A few case studies have suggested that B19V is related to meningitis and encephalitis (Okumura et al. 1993, Suzuki et al. 1995, Barah et al. 2001, Bonvicini et al. 2008, Douvoyiannis et al. 2009). Also associations between acute B19V infection and encephalopathy, peripheral neuropathy (Umene et al. 1995, Douvoyiannis et al. 2009), prenatal stroke (Craze et al. 1996, de Haan et al. 2006), and chronic fatigue syndrome have been reported (Kerr et al. 2002).

### 2.2.6.9. Other diseases

B19V infection has been linked to autoimmune disorders. An association between B19V infection and some diseases such as Kawasaki's disease (Nigro et al. 1994), Wegener's granulomatosis, polyarteritis nodosa (Harel et al. 2000), vascular purpura/vasculitis (Schwarz et al. 1989a, Dingli et al. 2000, Linton et al. 2000), glomerulonephritis (Wierenga et al. 1995), uveitis (Maini et al 1999, Heinz et al. 2005), and Raynoud's phenomenon (Harel et al. 2000) has been suggested, but the etiologic role of the virus in these diseases is controversial. In children, who were investigated or treated for various malignancies or cytopenias, B19V infection has been detected in 10% (Broliden et al. 1998, Yoto et al. 1993).

## 2.2.7. Definitions of pregnancy outcomes

In this doctoral thesis fetal loss is defined as intrauterine fetal death (IUFD) having occurred after 22 completed gestational weeks, and as miscarriage having occurred earlier. Of all clinically diagnosed pregnancies, approximately 10% are supposed to end in miscarriage. Reliable information on early pregnancy loss (subclinical miscarriages) is not available. However, it has been speculated that up to 75% of all human conceptions could end in early loss (Wilcox 2010).

According to the Finnish Medical Birth Register (MBR), 0.3% of all births end in stillbirth: intrauterine fetal death having occurred after 22 completed gestational weeks or having a birth weight of ≥500 g. In Finland, perinatal mortality – stillbirth or neonatal death during the first week of life – is <0.5% being among the lowest in the world. The prevalence of births with major anomalies (the International Classification of Diseases, ICD-9, codes 740–759) has been on average 3%. Multiple-birth deliveries account for 1.5% of all deliveries. Preterm births – birth before the 37th week of gestation – account for approximately 6% of all births. Low-weight births – birth weight <2500g – represent 4% of all births.

## 2.2.8. Parvovirus B19 infection during pregnancy

### 2.2.8.1. Seroprevalence and incidence of B19V infection

Of fertile-aged women 35–81% are B19V-seropositive, and thus immune (Gratacós et al. 1995, Skjöldebrand-Sparre et al. 1996, Valeur-Jensen et al. 1999, Jensen et al. 2000, Alanen et al. 2005, van Gessel et al. 2006, Mossong et al. 2008). Incidence values of B19V infections among susceptible pregnant women have been reported in six studies (Table 2). Also, in one study, prevalence and incidence rates were connected, when the mothers with B19V IgM-positive results in the beginning of the pregnancy were combined with the seroconverted mothers (Jensen et al. 2000).

Two of these studies included only non-epidemic periods. In a Swedish study (Skjöldebrand-Sparre et al. 1996), the seroprevalence was 81%, and 6.8% of the mothers seroconverted; all of these women gave birth to healthy infants. One mother with no change in antibody levels, but with B19V DNA-positive serum and placental samples, had intrauterine fetal death at 37 gestational weeks. In a Finnish study (Alanen et al. 2005), the seroprevalence was 59%. Neither maternal age nor number of previous children was related to the seroprevalence. The annual incidence was 1.5%. Two out of three B19V-seroconverted mothers worked with children.

Table 2. B19V seroprevalence and incidence values (IgG seroconversions) among pregnant women in different European countries.

| Study | Country | Study period | Population | Seroprevalence | Incidence during pregnancy |
|---|---|---|---|---|---|
| | | | N | % | % |
| **Non-epidemic period** | | | | | |
| Skjöldebrand-Sparre et al. 1996 | Sweden | 5/1990–10/1991 | 457 | 81 | 6.8 |
| Alanen et al. 2005 | Finland | 6–8/2000 | 558 | 59 | 1.3 |
| **Information on epidemics not available** | | | | | |
| Gratacós et al. 1995 | Spain | NA | 1610 | 35 | 2.9 |
| Mossong et al. 2008 | Belgium | NA | NA | 74 | 0.6 |
| | England& Wales | NA | NA | 62 | 0.7 |
| | Finland | NA | NA | 56 | 1.2 |
| | Italy | NA | NA | 60 | 0.9 |
| | Poland | NA | NA | 63 | 1.6 |
| **Non-epidemic and epidemic periods** | | | | | |
| Valeur-Jensen et al. 1999 | Denmark | 11/1992–6/1994 | 30946 | 65 | 2.4 |
| van Gessel et al. 2006 | Netherlands | 2/1998–6/2000 | 2567 | 70 | 2.4 |
| **IgG seroconversion and/or IgM seropositivity in the beginning of the pregnancy** | | | | | |
| Jensen et al. 2000 | Denmark | 11/1992–2/1994 | 3151 | 66 | 10.3 |

NA=not available

In a Spanish study (Gratacós et al. 1995) the seroprevalence was 35% and the incidence was 2.9%. Of the B19V-infected mothers 8.3% (5/60) had miscarriage during 9–19 weeks of gestation, while the others delivered healthy infants. Of the B19V-infected mothers 70% were asymptomatic. Mossong et al. (2008) reported 56–74% seroprevalences in different European countries. Estimated incidence rates were 0.6–1.6%. In these two studies information on epidemic or non-epidemic periods was not available.

In a Danish study (Valeur-Jensen et al.1999), the seroprevalence was 65%. It was correlated with an increasing number of siblings, having a sibling at the same age, parity, and occupational exposure to children. Of the mothers 2.4% seroconverted during pregnancy. The annual incidences were 1.5% during non-epidemic, and 13.0% during epidemic periods. Seroconversion rate increased along with increasing number of children, and was highest with mothers of children aged five to seven years. Nursery school teachers had a three-fold risk to have an acute B19V infection as compared to other pregnant women.

In a Dutch study (van Gessel et al. 2006), the seroprevalence was 70%. Women with low socioeconomic status and those professionally involved with children were more likely to be seropositive. Incidence was 2.4% during 1998–2000, including the B19V epidemic year 1998. None of the fetuses of the B19V-seroconverted mothers had reportedly signs of hydrops or congenital anomalies.

In a Danish study (Jensen et al. 2000), the seroprevalence of B19V IgG was 66%. Contrary to the other studies, the mothers with IgM-positive results indistinguishable of total calculations were included in the seroconverted mothers. Altogether, 10.3% of the mothers had evidence of B19V infection during pregnancy. The proportion increased significally from 1% to 13.5% before and during the epidemic, respectively. Children at home, serious medical diseases and a stressful job were related to increased risk of B19V infection. However, no increased risk was observed among women working with children, but the seroprevalence of B19V IgG was higher among women working with children compared to those with no occupational contact with children. Acute B19V infection during pregnancy was associated with miscarriages and IUFDs.

The incidence studies of B19V infection are not directly comparable, because of differencies in the length of following-up periods, inclusion criteria, and epidemic or non-epidemic periods. The annual incidences (IgG seroconversions) seem to be 1.5% during non-epidemic time (Alanen et al. 2005, Valeur-Jensen et al. 1999), and 13% during an epidemic time (Valeur-Jensen et al. 1999). Skjöldebrand-Sparre et al. (1999) found the highest incidence rate, although their study period covered no epidemics; a small number of B19V-seronegative women could probably be one reason for the high incidence in the study.

#### 2.2.8.2. Vertical transmission

B19V can be transmitted across the placenta during maternal infection. A vertical transmission rate of 25–50% has been reported (PHLS 1990, Gratacos et al. 1995, Koch et al. 1998, Miller et al. 1998, Yaegashi et al. 1998). These may be underestimated incidences of transmission, because fetuses infected in the beginning of pregnancy may fail to raise a measurable immune response. Later in gestation the transplacental transmission rate increases (PHLS 1990, Gratacos et al. 1995, Miller et al. 1998). In a study of Miller et al. (1998) 11%, 30% and over 60% of children, whose mothers had B19V infection during the first, second or third trimester, respectively, were B19V-IgG positive at the age of one indicating intrauterine fetal infection.

#### 2.2.8.3. Fetal anemia

Rh immunization is the major cause for the fetal anemia, whereas the other mechanism includes α-thalassemia, twin-to-twin transfusion, feto-maternal transfusion and infectious reasons. B19V is the main infectious cause of fetal anemia, but also other infections, such as CMV and *Toxoplasma gondii* can occasionally lead to anemia.

The fetus is most vulnerable for anemia during the first and second trimesters. Fetal erytropoiesis begins in the yolk sac at gestational week three, transferring to liver at week six, and finally to bone marrow at week 30 (Niskanen 1996). During the hepatic stage of hematopoiesis fetuses may be severely affected by B19V infection, because of the short life of their red blood cells (45–75 days), and a rapidly expanding red cell volume. A contributory factor of fetal anemia can also be the relative immaturity of the erythropoietic system and the fetal immune response. From the third trimester, with a more developed immune system, the fetus may more efficiently defend against B19V. A reduction in the globoside content of cells on the villous trophoplast layer of the placenta late in gestation has been described, and supposed to be the probable reason for the difference in fetal outcome during pregnancy (Jordan et al. 1999).

#### 2.2.8.4. Hydrops fetalis

The cause of hydrops fetalis – generalized odema of fetus – can be classified to immune and nonimmune types. The majority of immune hydrops fetalis results from Rh immunization. However, the number of these cases has decreased dramatically, because of prevention of maternal isoimmunisation. At present, the most common cause of hydrops fetalis is non-immune (Ismail et al. 2001) including chromosomal defects, cardiovascular, hematologic, and infectious-based causes, such as CMV, rubella virus, herpes simplex virus, *Toxoplasma gondii*, and B19V. Nevertheless, hydrops fetalis is an uncommon complication of pregnancy occurring in only 1/2500–3500 deliveries (Holzgreve et al. 1991).

B19V has been detected as a cause of hydrops fetalis in 8–17% of cases (Morey et al. 1992, Mark et al. 1993, Rogers et al. 1993, Schwarz et al. 1993, Jordan et al. 1996, Ismail et al.

2001). Development of hydrops after maternal B19V infection has been reported in 4–24% of cases (Enders et al. 1990, Schwarz et al. 1990, Searle et al. 1998b, Enders et al. 2004, Simms et al. 2009), but in some small studies no hydropic fetuses were observed (Rodis et al. 1990, Harger et al. 1998, Koch et al. 1998). B19V-associated hydrops fetalis and fetal death occur typically during the second trimester (Gray et al. 1986, Anand et al. 1987, Schwarz et al. 1988 and 1991, Morey et al. 1992, Yaegashi et al. 1994 and 1998, Nyman et al. 2002, Enders et al. 2004, Enders et al. 2010), but occasionally also during the first trimester (Tolfvenstam et al. 2001, Nyman et al. 2002). Mostly, these B19V-infected fetuses have succumbed when hydrops has been diagnosed.

Several possible pathogenic mechanisms for development of B19V-related hydrops fetalis have been described. Severe fetal anemia can lead to significant tissue hypoxia followed by a compensatory increase in cardiac output and hydrops. B19V is also able to infect directly fetal myocardial cells and to cause myocarditis (Weiland et al. 1987, Porter et al. 1988, Naides et al. 1989, Katz et al. 1990, Morey et al. 1992). The fetal immune system is immature making it vulnerable to infectious diseases. Liver dysfunction due to hemosiderosis, increased extramedullary hematopoiesis or direct infection of hepatocytes can also lead to hydrops fetalis (Metzman et al. 1989).

#### 2.2.8.5. Fetal outcomes of B19V-infected mothers

Among B19V-infected mothers, the excess risk of an adverse fetal outcome has been estimated to be 6–11% during the first half of gestation, and very low thereafter (PHLS 1990, Miller et al. 1998, Enders et al. 2004). Of the B19V-associated fetal deaths, 80% occurs within four weeks after maternal infection (Enders et al. 2004), and almost all within 12 weeks (Bond et al. 1986).

In an English study, the excess risk of fetal loss due to B19V infection was estimated to be 9% among B19V-infected women (PHLS 1990). Almost all the mothers with fetal loss were infected before gestational week 20. Six of 14 available fetal tissue samples were B19V-DNA positive. The morphological picture was reported in three of these six fetuses; only one fetus was hydropic. Most of the survived infants were followed up at least one year, but no appreciable anomalies were observed. This study was later expanded by Miller et al. (1998). During the first half of the pregnancy, the excess risk of fetal loss with B19V-infected women was still 9%, when compared to a control group with varicella infection; women with of without varicella has been reported to have a similar rate of the fetal loss (Pastuszak et al. 1994). The difference was most marked between 9–16 gestational weeks. Between 9–20 gestational weeks the overall risk of hydrops fetalis was 2.9%. No late effects of B19V infection were found in children followed for 7–10 years.

In a German study (Schwarz et al. 1990), 24% (18/76) of B19V-infected women had hydropic fetuses; 84% (15/18) of the hydropic fetuses deceased. Three fetuses had been treated by intrauterine transfusion, and they survived.

**Table 3. Fetal outcomes of mothers with serologically confirmed B19V infection during pregnancy.**

| Study | Study period (country) | Materials | Fetal deaths | | | | Hydrops | |
|---|---|---|---|---|---|---|---|---|
| | | | All | | Miscarriages | IUFDs | | |
| | | N | N | % | N | N | N | % |
| PHLS 1990 | 1/1985–6/1988 | 186 | 29 | 16 | 28 | 1 | 1 | 0.5 |
| Miller et al. 1998 | 6/1992–6/1995 (England) | 244 | 29 | 12 | 29 | None | 6 | 2.5 |
| | Combined cohorts | 430 | 58 | 14 | 57 | 1 | 7 | 1.6 |
| Enders et al. 2004 | 1/1993–12/1998 (Germany) | 1018 | 64 | 6 | 58 | 6 | 40 | 3.9 |
| Schwarz et al. 1990 | NA (Germany) | 76 | 15 | 20 | 8 | 7 | 18 | 24 |
| Rodis et al. 1990 | 12/1987–12/1988 (United States) | 39 | 2 | 5 | 2 | None | None | |
| Harger et al. 1998 | 1990–1996 (United States) | 52 | None | | | | None | |
| Chisaka et al. 2006 | 1999–2004 (Japan) | 100 | 6 | 6 | 5 | 1 | 3 | 3.0 |
| Koch et al. 1998 | NA (United States) | 43 | None | | | | None | |

IUFD = intrauterine fetal death

Three small follow-up studies have been made in the USA. Of B19V-infected women Rodis et al. (1990) found 5.1% (2/39) to have miscarriages, while others delivered healthy infants. None of the fetuses developed hydrops. Harger et al. (1998) studied pregnant women, who were exposed to B19V, and tested them serologically 10–14 days after exposure; 52 had an acute B19V infection. The main source of exposure was a child their own. One third of the B19V-infected mothers were asymptomatic. None of the fetuses of the B19V-infected women developed hydrops. All fetuses also survived. Koch et al. (1998) followed 43 B19V-infected women to delivery. All of these mothers delivered healthy infants at term and none of the fetuses was hydropic. Overall, 51% of fetuses had evidence of congenital B19V infection.

In a German study (Enders et al. 2004), among B19V-infected women the observed rate of miscarriages and IUFDs were 5.7% (58/1018) and 0.6% (6/960), respectively. Fetal death was only detected when the mother had an acute B19V infection during the first 20 gestational weeks. Of the fetal deaths 80% occurred within four weeks of acute maternal B19V infection. The overall prevalence of hydrops fetalis was 3.9% (40/1018) with a maximum of 7.1% if maternal B19V infection occurred between 13 and 20 gestational weeks. Of 40 hydropic fetuses 23 were studied and found to be B19V-DNA positive either in fetal blood or amniotic fluid. Of fetuses with severe hydrops following fetal cord blood transfusion 85% survived.

Three of six IUFDs were hydropic; one of these fetal deaths occurred due to accidental cord strangulation; one fetal death could not be further studied because of severe maceration; one fetus succumbed 25 weeks after maternal B19V infection. In this study a B19V-associated excess risk of fetal death was observed only during the first half of pregnancy, with a maximum during 13–16 gestational weeks.

In a Japanese study (Chisaka et al. 2006), the incidence of fetal deaths related to maternal B19V infection was 6%. All mothers of these fetuses were infected before 20 gestational weeks and tended to be symptomatic with rash and fever. The most common infectious source of the mothers was their own child.

In these above-mentioned, follow-up studies almost all fetal deaths occurred with maternal B19V infection during the first half of pregnancy. However, an association between early pregnancy loss and B19V infection could not be studied. On the other hand, in the three small studies no risk was found (Rodis et al. 1990, Harger et al. 1998, Rodis et al. 1998). In these follow-up studies, recruitment is different, and thus a direct comparison between studies may not be appropriate.

In the follow-up studies a sampling bias may be present, because only mothers with suspicious symptoms and/or contacts were studied. One third of adults are symptomfree, and thus probably out of follow-up. Also, symptoms of B19V infection are usually like in common cold, and B19V infection is not suspected. Little is known of differencies in pregnancy outcomes among women with or without symptoms. The proportion of

asymptomatic B19V infections has been reported to be higher among pregnant than non-pregnant women (Adler et al. 1993, Smoleniec et al. 1994a, Gratacos et al. 1995). Also, more adverse pregnancy outcomes have been observed among asymptomatic than symptomatic women in some (Schwarz et al. 1991, Smoleniec et al. 1994a), but not in all studies (Miller et al. 1998, Enders et al. 2004).

#### 2.2.8.6. Prevalencies of B19V infection among miscarriages and IUFDs

The prevalencies of B19V infection among IUFDs, but sometimes also among miscarriages, have been reported in five Swedish studies from the same research group (Table 4). During 1992–1998, including two major B19V epidemics, Skjöldebrand-Sparre et al. (2000) studied freshly frozen placental tissue samples of dead fetuses. Of them 8% (7/93) were B19V-DNA positive during or after 28 gestational weeks; one of the control placentas (1/50) was also B19V-DNA positive. All available FFPE blocks of these seven B19V DNA-positive cases were studied; six of seven placental tissues were positive. One of three fetuses with available tissue samples was B19V-DNA positive. None of the seven IUFDs were hydropic. None of the seven mothers had experienced clinical signs of B19V infection during pregnancy, and five maternal serum samples were B19V-IgG negative at birth.

Tolfvenstam et al. (2001) studied freshly frozen placental, fetal heart, lung, and liver tissue samples of IUFDs during non-epidemic time, 1/1998–5/1999, in Stockholm. B19V DNA was detected in 5% of miscarriages, in 15% of IUFDs, but in none of induced abortions. Two mothers of seven IUFDs had evidence of an acute B19V infection at the time of birth during the second-trimester. They had also B19V inclusion bodies, and immunohistochemistries were specific for B19V in the placentas. One of these two mothers had the only hydropic fetus in this study. In the other cases, three of four available placental tissues were not B19V-DNA positive, although in each case one fetal tissue was positive. In one case the placental tissue sample was found to be B19V-DNA positive, but fetal tissue samples were not. In one case placental tissue was not available, but one fetal tissue sample was B19V-DNA positive.

Norbeck et al. (2002) studied FFPE tissue samples of IUFDs during 2/1993–8/1997, and detected B19V DNA in 14% (13/92). The control group – placental biopsies from 60 normal pregnancies at term – contained no B19V DNA. Of ten studied placental tissue samples, six were B19V-DNA positive, and three of these had positive fetal tissue samples. However, all four mothers with DNA-negative placental samples had B19V DNA-positive fetal samples. Only two fetuses were hydropic; both had B19V DNA-positive placentas and fetal tissues during gestational weeks 24 and 28. Only one of the mothers studied with DNA-positive fetal samples (placenta was not studied) without hydrops was mentioned to have serological evidence of acute B19V infection.

**Table 4. B19V DNA in autopsied placental and/or fetal tissue samples.**

| Reference | Study period | Matherials | | B19V DNA-positive fetal deaths | | | Hydrops with B19V DNA-positive samples | Evidence of acute maternal B19V infection |
|---|---|---|---|---|---|---|---|---|
| | | | | All | Placentas | Fetal tissues | | |
| | | | N | N (%) | N | N | N | N |
| **Skjöldebrand - Sparre et al. 2000** | 1992–1998 | Placental tissues: | | | | | | |
| | | IUFDs | 93 | 7 (8) | 7 | 1 | 0 | 1 |
| | | Controls | 50 | 1 (2) | 1 | NT | 0 | NT |
| **Tolfvenstam et al. 2001** | 1/1998–5/1999 | Placental and/or fetal tissues: | | | | | | |
| | | Miscarriages | 37 | 2 (5) | 0 | 2 | 0 | 1 |
| | | IUFDs | 47 | 7 (15) | 3 | 5 | 1 | 2 |
| | | Controls | 53 | 0 (0) | 0 | NT | 0 | NT |
| **Norbeck et al. 2002** | 2/1993–8/1997 | Placental and/or fetal tissues: | | | | | | |
| | | IUFDs | 92 | 13 (14) | 6 | 10 | 2 | 1 |
| | | Controls | 60 | 0 (0) | 0 | NT | 0 | NT |
| **Nyman et al. 2002** | 3/1997–8/1999 | Placental tissues: | | | | | | |
| | | I trimester | 36 | 1 (3) | 1 | NT | NT | 0 |
| | | II trimester | 64 | 8 (13) | 8 | NT | NT | 6 |
| | | Controls | 53 | 0 (0) | 0 | NT | NT | 0 |
| **Petersson et al. 2004** | 1/1998–2/2001 | Placental tissues, fetal blood and/or amniotic fluid: | | | | | | |
| | | IUFDs | 52 | 2 (4) | 2 | 1 | 1 | 2 |
| | | Controls | 53 | 0 (0) | 0 | NT | NT | 0 |

IUFD = intrauterine fetal death; NT = not tested; Controls were placental tissues from normal pregnancies in term.

Nyman et al. (2002) studied freshly frozen placental tissue samples of deceased fetuses from the first and the second trimesters during 3/1997–8/1999. B19V DNA was found in 3% (1/36) of the samples from the first trimester. However, the corresponding maternal sera had no signs of acute B19V infection (IgG positive, IgM negative). B19V DNA was detected in 12% (8/64) of samples from the second trimester; range 14–25 gestational weeks, median 18. All six available serum samples of these mothers tested were B19V-IgG and IgM positive indicating an acute B19V infection. The control group – placental biopsies from 53 normal pregnancies at term – contained no B19V DNA. In this study, B19V DNA was not studied from fetal tissue samples.

Petersson et al. (2004) studied freshly frozen placental biopsies, fetal blood and/or amniotic fluid from IUFDs during 1/1998–2/2001. B19V DNA was observed in 3.8% (2/52) of placental biopsies, but not in 53 placentas from normal pregnancies at term. In these two B19V DNA-positive placentas immunohistochemistries were positive for B19V, and inclusion bodies were found in fetal tissue samples. The other fetus had B19V-containing blood and amniotic fluid samples, while the other fetus was not tested. Mothers of these fetuses had evidence of an acute B19V infection (IgG positive, IgM positive).

Contrary to the general hypotheses, Tolfvenstam et al. (2001) and Norbeck et al. (2002) found most of B19V-related IUFDs in late pregnancy, particularly without signs of fetal hydrops. In many cases only placental tissue samples were B19V-DNA positive, while fetal tissue samples were not studied or remained negative. Also, in many of B19V DNA-positive IUFDs there were no evidences of acute or recent maternal B19V infections, or placental tissue samples were negative, although fetal tissue samples were positive. However, B19V DNA was found more often among IUFDs and miscarriages than among control placentas. This indicates that B19V infections could have caused most of the fetal deaths with B19V DNA-positive fetal tissues, if the results are true.

Prevalence studies of B19V infection have concentrated to IUFDs, because IUFDs are usually registered and the proportions of IUFDs can be calculated at the population level. Among IUFDs, a sampling bias is probably uncommon, because missed data is independent on B19V infection. Instead, proportions of miscarriages are unknown, because most miscarriages occur without mothers being aware of pregnancy. Because only few per cents of miscarriages could be included in the studies, sampling bias is very probable. Therefore, veritable prevalence rates of B19V DNA could not be detected among miscarriages.

#### 2.2.8.7. Twin pregnancy

In twin pregnancy, both fetuses may have intrauterine infection, but infection in only one fetus has also been described (Zerbini et al. 1993, Pustilnik et al. 1994). Schiesser et al. (2009) reported a diamniotic, dichorionic twin pregnancy, where both fetuses were B19V-infected; only the other fetus developed hydrops and suffered intrauterine fetal death, while the other fetus was asymptomatic, and survived.

#### 2.2.8.8. Congenital anomalies

Many animal parvoviruses are known to cause anomalies (Jordan et al. 1994), and B19V is also thought to play a causative role. B19V-related anomalies have been described in cardiac (Wang et al. 2004, Konstantinidou et al. 2007), nervous (Rodis et al. 1988, Katz et al. 1996), genitourinary (PHLS 1990, Konstantinidou et al. 2007), digestive (Tiessen et al. 1994), and ocular (Weiland et al. 1987, Hartwig et al. 1989, van Elsacher-Niele et al. 1989, Plachouras et al. 1999, Busby et al. 2005) systems. The studies of B19V-associated anomalies have usually been case reports, and there is no firm evidence on teratogenicity. Exposure to B19V may be coincidental. However, if certain abnormalities are linked to B19V infection then the incidence of anomalies among all B19V-complicated pregnancies is less than 1% (Pattison 1994, Miller et al. 1998). B19V has normally a strong predilection for erythroid precursor cells, but it may be capable of infecting young immature fetal tissues (Hartwig et al. 1989). Intrauterine B19V infection may influence fetal organogenesis and development, and fetal damage may vary with gestational age of infection. Precise incidence of fetal adverse outcomes requires investigations in large, prospective studies of even more than 10 000 B19V-infected pregnant women.

### 2.2.9. Diagnosis

#### 2.2.9.1. Antibody assays

In B19V infection, the humoral immune response such as the production of neutralizing antibodies to the capsid proteins is thought to play a major role in limiting infections in humans. In general, the capsid proteins of parvoviruses have been shown to represent a major target for the humoral immune response. IgM antibodies against the structural proteins VP1 and VP2 are the first serological markers of an acute B19V infection and may be detected typically from day 10 to 2–3 months after contact (Anderson et al. 1985, Enders et al. 2006), but persistence for as long as nine months has been observed (Searle et al. 1998a). IgM antibodies against NS1 may take over six weeks to develop after onset of illness, which explains the lower prevalence of anti-NS1 antibodies in persons with acute B19V infection (Searle et al. 1998b). IgG antibodies may be detected about two to three weeks after infection and they can persist life-long, and protect against reinfection. Waning of B19V immunity may happen in the older population (Röhrer et al. 2008). The newborn infants may be protected by maternal IgG antibodies, which are able to cross the placenta. Of note, maternal IgM antibodies are too large to cross the placental barrier. Therefore, IgM in newborns is a marker of a congenital infection. The B19V-IgG seroprevalence has been shown to be >60% at birth, but decline to ≤20% around the age of one year (Eis-Hübinger et al. 1998, Miller et al. 1998, Enders et al. 2007). Antibody production is correlated with the disappearance of virus from the blood. In patients with a defect of immunoglobulin production persistent infection is possible (Kurtzman et al. 1989).

B19V serology can be determined using e.g. enzyme immunoassay (EIA), radioimmunoassay (RIA), or immunofluorescence (IF). B19V-specific antibodies are measured in standardized commercial solid-phase, enzyme-labelled immunoassays, usually with the use of recombinant capsid proteins. Most B19V antigens are produced in insect cell lines with recombinant baculovirus (Brown et al. 1990 and 1991, Kajigaya et al. 1991).

Supplementary serological assays such as VP1-IgG avidity EIA and VP2-IgG-ETS-EIA are advisable for strengthening the reliability of B19V serodiagnosis (Söderlund et al. 1995a and 1995b, Kaikkonen et al. 1999 and 2001). IgG specific for VP1 and for conformational epitopes of VP2 persist for life, whereas IgG recognizing linear epitopes of VP2 are only expressed during the acute phase of B19V infection. The time of primary infection can also be estimated by measurement of IgG avidity (Hedman et al. 1993, Söderlund et al. 1995a). Registration of an increase in IgG antibody titer in paired sera is also used in diagnosis of an acute B19V infection. B19V capsid antigens can also be detected, but this method is rarely used.

#### 2.2.9.2. DNA detection

Polymerase chain reaction (PCR) has increased the sensitivity of DNA detection in serum and tissue samples. However, increased sensitivity has brought along a great propensity for contamination. DNA tests are required to diagnose acute aplastic crisis and persistent infection, since antibody production is absent or minimal. Acute B19V infection can be diagnosed earlier and longer by PCR than by serological tests. Therefore, both IgM and viral DNA detection from serum has been recommended to be used to detect an acute or recent B19V infection (Erdman et al. 1991, Török et al. 1992), if avidity or ETS tests are not available. Hybridization of PCR products improves sensitivity and specificity, and confirms the results. However, PCR test alone, without serological tests, is not reliable, because it can be positive for several months after acute infection (Enders et al. 2006).

#### 2.2.9.3. Histology

Intranuclear inclusion bodies – also called lantern cells – have been linked to B19V infection. They have been demonstrated in erythroid precursor cells (Burton et al. 1986, Anand et al. 1987, Carrington et al. 1987) even when extensive autolysis was present (Burton et al. 1988, Rogers et al. 1993). The other cytological findings typical for B19V infection include giant pronormoblasts, cytoplasmic vacuolization, or immature chromatin (Gray et al. 1987, Caul et al. 1988, Schwarz et al. 1990, Morey et al. 1992, Rogers et al. 1993). Vasculitis and erythroblasts within placenta or inflammatory changes in the myocardium could raise a suspicion of B19V infection (Morey et al. 1992). Histological changes of fetal B19V infection seems to be similar across a range of gestational ages (Morey et al. 1992).

### 2.2.9.4. Maternal and fetal investigations during pregnancy

The most reliable diagnostics for primary B19V infection during pregnancy – and at the time of fetal hydrops – comprise serology (IgM and IgG, together with the VP2-IgG ETS and VP1-IgG-avidity assays) complemented with PCR of maternal serum (Enders et al. 2006 and 2008). Fetal diagnosis based on the detection of maternal IgG and IgM antibodies alone, can sometimes be unreliable. At the time of detecting hydrops fetalis, the maternal B19V IgM level may already have dropped below the detection limit; according to Enders et al. (2008) 15% of pregnant women were B19V IgM EIA-negative at the time of hydrops fetalis. It has also been speculated, that maternal IgG and IgM levels may be present at an undetectable level as a consequence of the physiological immunodepression in pregnancy or low viral antigen stimulation (Bonvicini et al. 2009). PCR analysis of maternal blood along with serology appears to identify B19V infection with great diagnostic value.

After maternal B19V infection almost all fetal complications occur within 12 weeks (PHLS 1990, Enders et al. 2004, Simms et al. 2009). Thereby, in all patients with strong suspicion of an acute B19V infection during pregnancy, weekly ultrasound examinations for up to 12 weeks after maternal exposure should be performed. Sonografically detectable markers of infection include pericardial or pleural effusions, ascites, abdominal wall odema, polyhydramnios, and decreased fetal movements.

Both fetal cord blood samples and amniotic fluid samples are suitable for diagnosis of fetal B19V infection, but the detection of B19V DNA in the cells of amniotic fluid samples proved to be the most reliable diagnostic system (Bonvicini et al. 2009). However, these procedures include the risk of fetal demise. Fetal IgM may also be undetectable due to the immaturity of the fetal immune system at the early stage of pregnancy.

The riskless way to investigate moderate to severe fetal anemia in its early stages before the appearance of hydrops fetalis, is to measure fetal middle cerebral artery peak systolic velocity (MCA-PSV) (Mari et al. 2000). Fetal anemia is associated with decreased blood viscosity leading to increased venous return and preload with consequent increase in cardiac output. This response leads to increased blood flow to vital organs: the brain, heart and adrenal glands. This is observed as increase in fetal MCA-PSV. MCA is the main branch of the circle of Willis, and the direct continuation of the internal carotid artery. It supplies 80% of the blood flow to the cerebral hemisphere (Hernandez-Andrade et al. 2004). The peak velocity of the MCA increases significantly with gestational age.

The value of serum alpha-fetoprotein in the diagnosis and the prognosis of fetal B19V infection remain controversial. Some studies have described an association between raised alpha-fetoprotein level and poor prognosis of fetus (Carrington et al. 1987, Bernstein et al. 1989, Smoleniec et al. 1994b), while others have not found any correlation (van Elsacher-Niele et al. 1989, Saller et al. 1993, Johnson et al. 1994, Komischke et al. 1997, Simms et al.

2009). However, alpha-fetoprotein is raised for several reasons, and it could only be suggestive for intrauterine B19V infection.

### 2.2.10. Treatment

There is no medicine or vaccine that prevents B19V infection. However, most of B19V-infected children and adults need no specific therapy, and symptomatic treatment is usually sufficient. Isolation of patients is not practical, except in hospitals with very contagious B19V-infected patients with aplastic anemia. Aplastic crisis in chronic hemolytic anemia patients may require erythrocyte transfusions. Non-steroidal anti-inflammatory drugs may be indicated for arthralgia.

Intravenous immunoglobin therapy has been described to be effective for the treatment of chronic infection in immunodeficient patients leading to cleareance of B19V viremia and resolution of symptoms (Koch et al. 1990). Immunoglobin treatment has had favourable outcome in arthritis (Stahl et al. 2000), vasculitis (Finkel et al. 1994), meningoencephalitis (Barah et al. 2001), and chronic fatigue syndrome (Kerr et al. 2003). Also, recovery of fetal anemia and resolution of hydrops fetalis after high-dose intravenous immunoglobin has been described in some (Selbing et al. 1995, Rugolotto et al. 1999), but not in all studies (Brown et al. 1994a).

Spontaneous resolution of hydrops fetalis secondary to B19V infection can occur (Morey et al. 1991, Pryde et al. 1992, Smoleniec et al. 1994b), but sometimes medical treatments are necessary. Intrauterine transfusion was first described as a therapy regimen in B19V-associated fetal anemia and hydrops in the 1980's (Hansmann et al. 1989). Intrauterine transfusion is predominantly performed as transfusion of packed red cells into the umbilical vein. The tranfused red cell volume depends on the weight and hemoglobin level of the fetus. Resolution of hydrops is supposed to occur in approximately three to eight weeks after intrauterine transfusion (Soothill et al. 1990, Odibo et al. 1998). Among B19V-infected anemic fetuses intrauterine transfusions decrease the amount of fetal deaths, and ≥75% of fetuses may survive (Fairley et al. 1995, Rodis et al. 1998a, Schild et al. 1999, Enders et al. 2004). Of untreated fetuses about half may survive (Fairley et al. 1995, Rodis et al. 1998b, von Kaisenberg et al. 2001, Enders et al. 2004), but no with severe hydrops (Enders et al. 2004). Rodis et al. (1998b) found that almost all of the fetal deaths after transfusion occurred within 48 hours suggesting that these fetuses were moribund at the time of transfusion or died as a result of procedure-related complications. Also, as a complication of prolonged posttransfusion, intra-amniotic bleeding after B19V-related thrombocytopenia has been reported (Peters et al. 1990).

### 2.2.11. Long-term outcome following intrauterine parvovirus B19 infection

Fetuses with intrauterine B19V infection and/or successful intrauterine transfusion have a good neurodevelopmental long-term prognosis according to most studies (Sheikh et al. 1992, Fairley et al. 1995, Cameron et al. 1997, Hudon et al. 1998, Miller et al. 1998, Rodis et al. 1998a, Dembinski et al. 2002), whereas some studies have shown mild-to-severe neurodevelopment delay (Nagel et al. 2007, Pistorius et al. 2008). At one year follow-up, no apparent increase was detected in the frequencies of developmental delays in 16 infants with exposure to B19V in utero (Sheikh et al. 1992, Fairley et al. 1995). Cameron et al. (1997) describes a case-report of an infant, who underwent repeated intrauterine transfusions and had a normal neurodevelopment at two years follow-up. Miller et al. (1998) found no late effects of intrauterine B19V infection in 129 children aged 7–10 years.

## 2.3. Human bocavirus

### 2.3.1. Morphology

Human bocavirus (HBoV) was first described in clinical specimens from children with respiratory tract infections in 2005 in Sweden (Allander et al. 2005). HBoV is a member of *Parvovirinae*, like B19V. HBoV is a nonenveloped, single-stranded, small DNA virus. The 5217-base viral genome contains three open reading frames: two encoding non-structural capsid proteins NS1, NP1, and one encoding viral capsid proteins (VP1-3) (Allander et al. 2005, Cecchini et al. 2009). VP1 and VP2 have identical sequences, except for an additional aminoterminal domain of 129 amino acids in VP1. The molecular masses of VP1, VP2 and VP3 are 72 kDa, 68 kDa and 62 kDa, respectively. The genetic variability of known HBoV strains is low (Allander et al. 2005). NS1 and NP1 genes are the most conserved areas, whereas small variations have been detected in VP1 and VP2. HBoV is closely related to bovine parvovirus (BPV) and minute virus of canines (CnMV), which are classified as belonging to the *Bocavirus* (i.e. bovine/canine) genus. HBoV has only 42% and 43% amino acid identity within the major open reading frames with these two viruses, respectively, and 18% amino acid identity to B19V VP1 (Allander et al. 2005).

### 2.3.2. Epidemiology

HBoV is prevalent worldwide, and has been detected in respiratory tract, blood and fecal samples. HBoV seroprevalence is shown to be lowest during four to eight months of age and to increase with advanced age (Endo et al. 2007, Kahn et al. 2008, von Linstow et al. 2008, Söderlund-Venermo et al. 2009). Most infants under six months of age have HBoV antibodies, which probably are maternally derived. Acute HBoV infection occurs most often in children from six months to two years of age. HBoV seroprevalence is >90% among

children at the age of over three years (Karalar et al. 2010). Almost all of the children are exposed to HBoV before school age (Endo et al. 2007, Don et al. 2009, Söderlund-Venermo et al. 2009).

Most studies have reported seasonal fluctuation of HBoV infection; most of the infections occur during winters (Allander et al. 2005, Arnold et al. 2006, Foulongne et al. 2006, Kesebir et al. 2006, Ma et al. 2006, Weissbrich et al. 2006, Chung et al. 2007, Fry et al. 2007, Lau et al. 2007, Pozo et al. 2007, Brieu et al. 2008, Canducci et al. 2008, Chow et al. 2008, Cilla et al. 2008, Smuts et al. 2008), while occasional studies have not found seasonality (Bastien et al. 2006 and 2007, Maggi et al. 2007, Zheng et al. 2009). A male predominance among HBoV DNA-positive children has been obtained in most studies (Allander et al. 2005, Arnold et al. 2006, Bastien et al. 2006, Foulongne et al. 2006, Kesebir et al. 2006, Ma et al. 2006, Sloots et al. 2006, Weissbrich et al. 2006, Allander et al. 2007, Chung et al. 2007, Lau et al. 2007, Qu et al. 2007, Völz et al. 2007, Chow et al. 2008, Christensen et al. 2008, Dina et al. 2009, Wang et al. 2010), but not in all (Maggi et al. 2007, Redshaw et al. 2007). Gender-specific differencies were not detected in the seroprevalence of HBoV IgG among children under three years of age (Karalar et al. 2010).

HBoV DNA has been occasionally found in adults (Bastien et al. 2006, Manning et al. 2006, Fry et al. 2007, Maggi et al. 2007, Chow et al. 2008, Longtin et al. 2008, Costa et al. 2009, Garbino et al. 2009, Ringhausen et al. 2009), often with immunosuppressing, cardiac or pulmonary diseases (Manning et al. 2006, Maggi et al. 2007, Chow et al. 2008, Longtin et al. 2008, Ringhausen et al. 2009). The presence of HBoV in immunosuppressed adults could be explained by reinfection, persistence or reactivation. Among healthy blood donors Lindner et al. (2008b) found 1% HBoV IgM-positivity, whereas Fryer et al. (2007) found no HBoV DNA-positive samples. HBoV IgG-positivity has been observed in >94% of adults (Endo et al. 2007, Lindner et al. 2008b, Söderlund-Venermo et al. 2009).

### 2.3.3. Persistence

Contrary to B19V, no evidence of lifelong tissue persistence of HBoV has been found. Although HBoV causes systemic infection, HBoV has not been detected in post mortem bone marrow, brain, or lymphoid tissues in HIV-infected persons indicating no persistence in these tissues (Manning et al. 2007). However, in a recent study HBoV DNA was found in 5% of cardiac tissue samples of patients, who underwent open-heart surgery (Kuethe et al. 2009). HBoV has also been shown to remain detectable in the nasopharynx of some individuals for up to six months after primary infection (von Linstow et al. 2008, Blessing et al. 2009).

### 2.3.4. Clinical manifestations

After B19V, HBoV is the second known parvovirus species pathogenic to humans. Adeno-associated viruses (AAV) are also parvoviruses which infect humans, but are generally considered non-pathogenic. However, associations between AAV and miscarriage have been speculated (Tobiasch et al. 1994). Furthermore, four other parvoviruses – PARV4, HBoV2, HBoV3, and HBoV4 – have recently been detected in human blood, tissues or stools, however, most with unknown clinical significance (Jones et al. 2005, Arthur et al. 2009, Kapoor et al. 2009 and 2010).

Respiratory infections

Respiratory syncytical virus (RSV), rhinovirus, adenovirus, influenza virus, and parainfluenza virus have been the most common causes of respiratory tract infections in children. The majority of these are RNA viruses. In addition to HBoV, the new respiratory infection-associated viruses – human metapneumovirus and new species of human coronavirus – have recently been discovered. HBoV-related clinical findings include wheezing, cough, fever, pneumonia, bronchiolitis, hypoxia, rhinitis and diarrhea. HBoV DNA has been detected frequently in 1–19% of respiratory specimens screened from children mostly under two years of age with upper or lower respiratory tract infections (Allander et al. 2005, Arden et al. 2006, Arnold et al. 2006, Manning et al. 2006, Sloots et al. 2006, Weissbrich et al. 2006, Allander et al. 2007, Chung et al. 2007, Maggi et al. 2007, Neske et al. 2007, Pozo et al. 2007, Qu et al. 2007, Redshaw et al. 2007, Vicente et al. 2007, Völz et al. 2007, Brieu et al. 2008, Canducci et al. 2008, Christensen et al. 2008, Cilla et al. 2008, Longtin et al. 2008, Smuts et al. 2008, Dina et al. 2009, Gagliardi et al. 2009, Ringhausen et al. 2009, Midulla et al. 2010, Wang et al. 2010), often in combination with peribronchial and pneumonic infiltrates. HBoV has also been detected in the middle ear fluids from children with acute otitis media (Beder et al. 2009, Rezes et al. 2009). HBoV tends to be more common in samples from the lower respiratory tract than in nasal specimens (Arnold et al. 2006, Brieu et al. 2008, Christensen et al. 2008, Karalar et al. 2010). However, most of the studies included mostly hospitalized persons with severe illnesses. The results of the studies are not exactly comparable, because of different study designs, technical methods, and regional and temporal differencies in the incidence of the HBoV infection.

Initially, HBoV was found significantly more frequently among children with acute respiratory symptoms than among asymptomatic children (Kesebir et al. 2006, Allander et al. 2007, Fry et al. 2007, Maggi et al. 2007) indicating a causality between HBoV and respiratory tract infections. However, recently HBoV DNA has been found also in asymptomatic children (von Linstow et al. 2008, Martin et al. 2009), pointing to persistent shedding or mucosal contamination (Söderlund-Venermo et al. 2009). Viremia of HBoV could not be shown in most of the studies, because serum was available only in a few studies. Allander et al. (2007) found that HBoV DNA in blood coincided with an ongoing symptomatic infection, and disappeared after recovery. HBoV-IgM positivity has been found approximately in 60% of

patients with HBoV DNA-positive nasal swabs (Karalar et al. 2010, Wang et al. 2010). The presence of IgM was significantly more prevalent in viremic patients and those diagnosed with a high load of DNA in respiratory specimens (Söderlund-Venermo et al. 2009, Wang et al. 2010).

HBoV causes upper and lower respiratory tract symptoms both with, and without other respiratory viruses. The rate of co-infection has been found in 18–91% of cases (Allander et al. 2005 and 2007, Arden et al. 2006, Sloots et al. 2006, Weissbrich et al. 2006, Chung et al. 2007, Fry et al. 2007, Lau et al. 2007, Pozo et al. 2007, Redshaw et al. 2007, Vicente et al. 2007, Völz et al. 2007, Brieu et al. 2008, Canducci et al. 2008, Christensen et al. 2008, Cilla et al. 2008, Gagliardi et al. 2009, Zheng et al. 2009, Wang et al. 2010), especially with RSV. However, the rate of coinfection is probably more common, because several respiratory pathogens were not tested for in the studies.

HBoV viral load has been significantly higher in samples from children with HBoV monoinfection than in those with coinfection in some (Allander et al. 2007, Brieu et al. 2008), but not in all studies (Neske et al. 2007, Zheng et al. 2009). Infants with dual infections has had a higher clinical severity score and more days of hospitalization than children with monoinfection (Cilla et al. 2008, Midulla et al. 2010). High viral loads are supposed to indicate acute primary infection (Allander et al. 2007, Kantola et al. 2008, Söderlund-Venermo et al. 2009) and to correlate with the severity of respiratory symptoms (Neske et al. 2007).

Gastroenteritis

Rotavirus, adenovirus, and calicivirus are major causes of acute gastroenteritis. Rotavirus is the most common cause of viral gastroenteritis during the first five years of age (Olesen et al. 2005). Recently, HBoV DNA has been detected in 1% to 9% of fecal samples of children with gastroenteritis (Albuquerque et al. 2007, Lau et al. 2007, Lee et al. 2007, Vicente et al. 2007, Yu et al. 2008, Szomor et al. 2009, Chow et al. 2010), and in 1.5% of those of adults (Chow et al. 2010). Of these cases 21% to 78% were coinfected with other intestinal pathogens (Arbuquerque et al. 2007, Lau et al. 2007, Lee et al. 2007, Vicente et al. 2007, Yu et al. 2008, Chow et al. 2009). Most of the cases occurred also in combination with acute respiratory tract infections; HBoV has been detected in 45% of fecal samples with HBoV-positive respiratory samples (Neske et al. 2007). Althoug HBoV is frequently detected in stools, its clinical significance in gastroenteritis is uncertain. However, another novel bocavirus, named HBoV2, has been associated with acute gastroenteritis in young children (Arthur et al. 2009, Kapoor et al. 2009).

Bocavirus infections during pregnancy

Parvoviruses infect bone marrow, respiratory and/or intestinal epithelium, and fetal tissues due to their need of proliferating host cells. In addition to B19V, the other parvoviruses, such

as porcine parvovirus, canine parvovirus, and the animal bocaviruses – BPV and CnMV – may also cause reproductive disorders (Carmichael et al. 1994, Manteufel et al. 2008).

During the first and the second trimester BPV can lead to death of the fetus with resorption, mummification and miscarriage. Likewise HBoV, BPV and CnMV have been associated with respiratory and enteric symptoms of young animals. Hydrops and intranuclear inclusions have been found in BPV-infected fetuses, like in B19V-infected fetuses. Enders et al. (2009) studied the prevalence of HBoV DNA in amniotic fluid samples of fetuses with hydrops, anemia, or isolated effusions, and found no positive result. Of note, anti-HBoV IgG antibodies were detected in 100% and 94% of serum samples of mothers with fetal hydrops and normal ultrasound findings, respectively, indicating immune response. The impact of HBoV infection on the pregnant woman and her fetus is still unknown.

### 2.3.5. Diagnosis

HBoV infection is systemic and viremic, and elicits B- and T-cell responses (Endo et al. 2007, Kantola et al. 2008, Lin et al. 2008, Lindner et al. 2008a and 2008b, Cecchini et al. 2009, Söderlund-Venermo et al. 2009, Kumar et al. 2011). HBoV infections can be diagnosed by quantitative PCR of respiratory tract secretions and/or serum, or by serology. However, PCR of nasopharyngeal aspirates alone is not sufficient, and accurate HBoV diagnosis requires serologic analysis or PCR of serum (Söderlund-Venermo et al. 2009). The human parvoviruses HBoV and B19V are antigenically distinct (Kahn et al. 2008), and no cross-reactivity has been observed between them (Endo et al. 2007, Kahn et al. 2008, Kantola et al. 2008, Lindner et al. 2008a).

### 2.3.6. Treatment

No medicine or vaccine is available against HBoV infection. For most of HBoV-infected children symptomatic treatment is usually sufficient. Most adults have HBoV-IgG antibodies, and they are probably safe from HBoV infection. However, secondary immune activations have been observed in immunocompetent adults (Hedman et al. 2010).

## 2.4. Risk factors of child-care employees for reproductive health

### 2.4.1. Infectious risk factors

Child-care employees are at increased risk for several infections, especially for upper and lower respiratory tract infections. Most of these infections are not harmful for pregnancy. Previously, morbilli, parotitis and rubella infections have been typical in childhood and have caused severe adverse pregnancy outcomes. Since 1982 MPR (morbilli, parotitis, rubella) vaccination has been included in the Finnish national vaccination program, and almost all of these infections have disappeared. However, immigrants can probably have these infections.

Parvovirus B19

The risk of B19V infection during pregnancy has been estimated to be greatest among child-care employees, school teachers and mothers tending B19V-infected children at home. Among child-care employees B19V seroprevalences are 50–80% (Gillespie et al. 1990, Cartter et al. 1991, Adler et al. 1993, Valeur-Jensen et al. 1999, Stelma et al. 2009; Table 5). Incidences have been 9–31% during epidemic, and 7% during non-epidemic times. According to a Danish study, child-care employees have even a three-fold increased risk and school teachers have a 1.6-fold increased risk for an acute B19V infection as compared to other pregnant women (Valeur-Jensen et al. 1999). Likewise, women in daily contact with school-aged children had a five-fold increased occupational risk for B19V infection as compared to employees of a hospital (Adler et al. 1993). In a study of child-care and school personnel during a community B19V epidemic, the risk for B19V infection was increased among the child-care employees and the teachers, who had contact with young children, and who had more symptomatic children in their classroom (Gillespie et al. 1990). The teachers had also a significantly higher B19V seroprevalence (62%) than other personnel (53%) (RR 1.2; 95% Cl 1.00–1.37). In another study of a B19V epidemic in a primary school, almost all B19V-susceptible adults were infected (Tuckerman et al. 1986). In a study of pregnant women with an exposure to B19V during an epidemic, 6% of susceptible women had serological evidence of recent B19V infection (Cartter et al. 1991). The highest infection rates were for schoolteachers (16%), child-care employees (9%) and homemakers (9%). Women working outside the home, but not in school or child-care centers had the lowest risk (4%). In a Canadian study, B19V seroprevalence increased with age and with working experience in child-care centers (Gilbert et al. 2005). An occupational risk was also indicated in a study of hydropic fetuses; one third of the mothers of these fetuses worked in child-care centers (Schwarz et al. 1988). A large B19V infection rate has also been detected among hospital staff during B19V epidemics (Bell et al. 1989). Two B19V-infected patients with sickle cell disease and aplastic crisis lead to attack rates of 36% and 38% among the susceptible hospital staffs.

In contrary to the before-mentioned studies with an increased occupational risk for B19V infection, Harger et al. (1998) found no statistically significant differences in eight categories

of maternal occupation including elementary school teachers, child-care and preschool teachers and homemakers. Likewise, no association was observed with B19V seropositivity and work at a child-care centre in the studies from the United States (Koch et al. 1989) and the Netherlands (Stelma et al. 2009).

Cytomegalovirus (CMV)

CMV infection is a typical childhood disease usually infecting children under three years of age. The virus is excreted to nearly all body fluids: urine, saliva, cervical and vaginal secretions, milk, semen, tears and blood. The infected children are able to excrete the virus even up to two years or longer (Pass et al. 1986b). About half of the children younger than three years of age attending child-care centers are likely to acquire CMV infection (Pass et al. 1984, Adler et al. 1988). The prevalence of CMV infection is three times higher among children attending child-care centers than among those cared for at home (Pass et al. 1984, Hutto et al. 1985).

CMV is the leading cause of congenital infection. Of child-care employees 30–60% has been seronegative, and annual seroconversion rates have ranged from 8% up to 20% (Adler et al. 1989, Pass et al. 1990, Murph et al. 1991, Ford-Jones et al. 1996, Jackson et al. 1996, Joseph et al. 2005, Stelma et al. 2009; Table 5). The rates have been higher than 2% in the comparison group (Adler et al. 1989). CMV seroconversion has been observed especially during the first two years of child-care employment (Stelma et al. 2009). Also parents of children attending child-care centers have increased rates of CMV infection (Pass et al. 1986a). About 40% of maternal CMV infections are vertically transmitted to the fetus (Peckham et al. 1991). Approximately 10–20% of these infants have a symptomatic CMV infection at birth, and additionally 5–15% will later develop symptoms (Gaytant et al. 2003) like neurologic diseases, mental retardation, deafness and/or blindness (Yinon et al. 2010). In general, about 0.5–2% of all newborns are infected by CMV in utero. The risk of an adverse pregnancy outcome is greatest among mothers, who are infected during the first half of pregnancy. No vaccination is available for CMV, but infection can be prevented effectively by giving information on the risks and on preventive procedures, like hand-washing (Adler et al. 2004, Joseph et al. 2006, Vauloup-Fellous et al. 2009) and gloves use (Ford-Jones et al. 1996).

Varicella zoster virus (VZV)

VZV infection is a typical childhood disease causing immunity, although the virus remains in neural ganglios, and can appear as shingles typically after fertile age. The seroprevalence of VZV among child-care employees is >90% (Lerman et al. 2004, Reigner et al. 2005). In general, there are annually approximately 30 maternal VZV infections in Finland (Mustonen et al. 1998). One fourth of fetuses, whose mother has VZV infection, are supposed to be infected (Enders et al. 1994). Infection during the first half of pregnancy can cause fetal damage in 1% of cases. Maternal infection with onset within a few days pre- or post-delivery can lead to life-threatening neonatal VZV. Vaccine has been recommended for all adults of

unknown evidence of past VZV infection. Because VZV vaccine does not yet belong to the general vaccine program, epidemics in child-care centers are typical in Finland. However, occupational risk for this infection is low, because of immunity of adult population.

Table 5. Seropositivities and seroconversion rates of various virus infections among child-care employees.

| Reference | Country | Population | Seropositive | Seroconversion rate |
|---|---|---|---|---|
| | | N | % | % |
| **Parvovirus B19** | | | | |
| Cartter et al. 1991 | USA | 42 | 48 | 9 [a] |
| Gillespie et al. 1990 | USA | 50 | 68 | 31 [a] |
| Adler et al. 1993 | USA | 2730 | 60 | 7 [b] |
| Valeur-Jensen et al. 1999 | Denmark | 390 | 78 | 7 [c] |
| Stelma et al. 2009 | Netherlands | 310 | 71 | NA |
| **Cytomegalovirus** | | | | |
| Adler et al. 1989 | USA | 610 | 41 | 11 [d] |
| Pass et al. 1990 | USA | 509 | 63 | 20 [d] |
| Murph et al. 1991 | USA | 252 | 38 | 8 [d] |
| Ford-Jones et al. 1996 | Canada | 206 | 67 | 13 [d] |
| Jackson et al. 1996 | USA | 360 | 62 | NA |
| Joseph et al. 2005 | Canada | 473 | 57 | NA |
| Stelma et al. 2009 | Netherlands | 310 | 57 | NA |
| **Varicella zoster virus** | | | | |
| Jackson et al. 1996 | USA | 360 | 98 | NA |
| Lerman et al. 2004 | Israel | 121 | 91 | NA |
| Reigner et al. 2005 | France | 241 | 100 | NA |

NA = not vailable; [a] = epidemic period; [b] = non-epidemic period; [c] = mixed period; [d] = annual

Influenza virus and fever

Influenza virus is effectively spread in garrisons, schools, and child-care centers. During the influenza pandemics of 1918 and 1957, and in the H1N1 pandemic in 2009, mortality rates appeared to be abnormally high among pregnant women (Rasmussen et al. 2008, Jamieson et al. 2009). Following influenza pandemics adverse pregnancy outcomes – miscarriages, preterm birth, and increase in defects of the central nervous system – have been reported

(Rasmussen et al. 2008). Oral clefts and eye anomalies, childhood leukemia, schizophrenia and Parkinson's disease have also been linked with seasonal influenza in some studies (Saxén et al. 1975, Takahashi et al. 2001, Busby et al. 2005, Brown et al. 2006, Kwan et al. 2007). In early pregnancy, hyperthermia is related to an increased risk of miscarriages, defects of the central nervous, cardiac systems and congenital renal anomalies (Kline et al. 1985, Edwards et al. 1995, Chambers et al. 1998, Graham et al. 1998, Botto et al. 2001, Abe et al. 2003, Moretti et al. 2005). Although high level fever has been shown to lead to adverse pregnancy outcomes, the effect of influenza virus on the fetus is not well understood.

### 2.4.2. Other risk factors

Child-care employees may be exposed to pronounced physical exertion – lifting, bending, stooping, squatting, carrying loads – which may influence intra-abdominal pressure, uterine blood flow, hormonal balance, and nutritional status, all of which are important determinants of embryonic and fetal development and survival (Ahlborg et al. 1995). Heavy lifting has been related to an increased risk of miscarriages, preterm delivery, and low birth weight in some (Saurel-Cubizolles et al. 1987, McDonald et al. 1988a, Armstrong et al. 1989, Ahlborg et al. 1990, El-Metwalli et al. 2001, Bonzini et al. 2007), but not in all studies. Increased risk of SGA (Tuntiseranee et al. 1998), low birth weight (Oths et al. 2001, Meyer et al. 2007), and preterm delivery (Croteau et al. 2007, Meyer et al. 2007) has also been linked to high job demands, which has been reported among child-care employees (Bright et al. 1999).

### 2.4.3. Pregnancy outcomes among child-care employees

Although child-care work is a common occupation with reproductive work hazards among fertile-aged employees, the relationship between child-care work and pregnancy outcome has rarely been investigated. In a small Swedish study, the occurrence of miscarriage was found to be higher in pregnancies during employment at child-care centers than in pregnancies in other types of work (Göethe et al. 1992). In the Canadian population-based studies the pregnancy outcomes in 60 different occupations were examined. The child-care employees, like the primary school teachers, had an elevated risk of congenital defects compared to the general population (McDonald et al. 1987). The infants of the child-care employees had an increased risk for a low birth weight and retardation in fetal growth (Armstrong et al. 1989), but not for preterm birth or an increased fetal mortality rate (McDonald et al. 1998a and 1998b).

## 2.5. Legislation

In Finland, mothers have the right to a maternity leave, which has to start not later than 30 business days (six business days per week) before the expected date of confinement, and end after 105 business days. After the maternity leave, the mother or father is entitled to a parental leave with a maximum length of 158 business days. Until the child is three years old one of the parents is allowed to stay at home to take care of the child.

In Finland, the legislation of special maternity leave has existed since 1991. A special maternity leave may be necessary, if the mother's job is considered to be hazardous to the development of the fetus. A Directive of the European Union refuses diving, mining, and being exposed to lead and to *Toxoplasma gondii* (seronegative pregnant women) at work during pregnancy. The law determines that pregnant women are not allowed to be exposed to some chemicals, biological and physical factors that might be reproductively harmful. The role of B19V in this schema is confusing. Although an increased risk of B19V infection during pregnancy has been observed in occupations where pregnant women work with children, a policy of routinely excluding pregnant women from high-risk workplace has not been generally recommended (CDC 1989, Morgan-Capner et al. 2002). However, in Germany, strict precautions have been brought into use. During the first 20 gestational weeks pregnant women are not allowed to work with children under six years of age; if reassignment of mother to other duties is impossible, withdrawal from work is mandatory (Enders et al. 2007). In Denmark, B19V-seronegative mothers occupationally exposed to B19V have to be removed to other duties for six weeks after the latest outbreak (the Danish Working Environment Authority). In Finland, pregnant B19V-susceptible health care employees are not recommended to care for patients with aplastic crisis, who may be highly contagious. During epidemics, e.g. in a child-care centre, the primary measure is reassignment of the mother to other duties, but if this is not practicable, a special maternity leave can be allocated.

# 3. AIMS OF THE STUDY

The general aim of the study was to create a scientific basis for human parvovirus infections in order to assess the occupational safety of pregnant child-care employees, and to formulate special maternity leave regulations in Finland. Two human parvoviruses – parvovirus B19 (B19V) and bocavirus (HBoV) – known to be pathogenic, were included in the study. B19V has been substantiated to cause adverse pregnancy outcomes, but the impact of a newly found parvovirus, HBoV, on pregnant women and fetuses was unknown.

The detailed objectives were

- To investigate the role of B19V DNA in miscarriages and intrauterine fetal deaths.

- To obtain new and internationally significant knowledge about the occurrence of the new HBoV DNA in the fetus and about its possible effects on pregnancy outcome.

- To investigate whether child-care personnel are at increased risk of adverse pregnancy outcome (i.e., preterm birth, low birth weight, smallness for gestational age, perinatal death and congenital anomalies).

- To investigate whether child-care personnel have an elevated risk of B19V infection.

# 4. MATERIALS AND METHODS

## 4.1. Study design

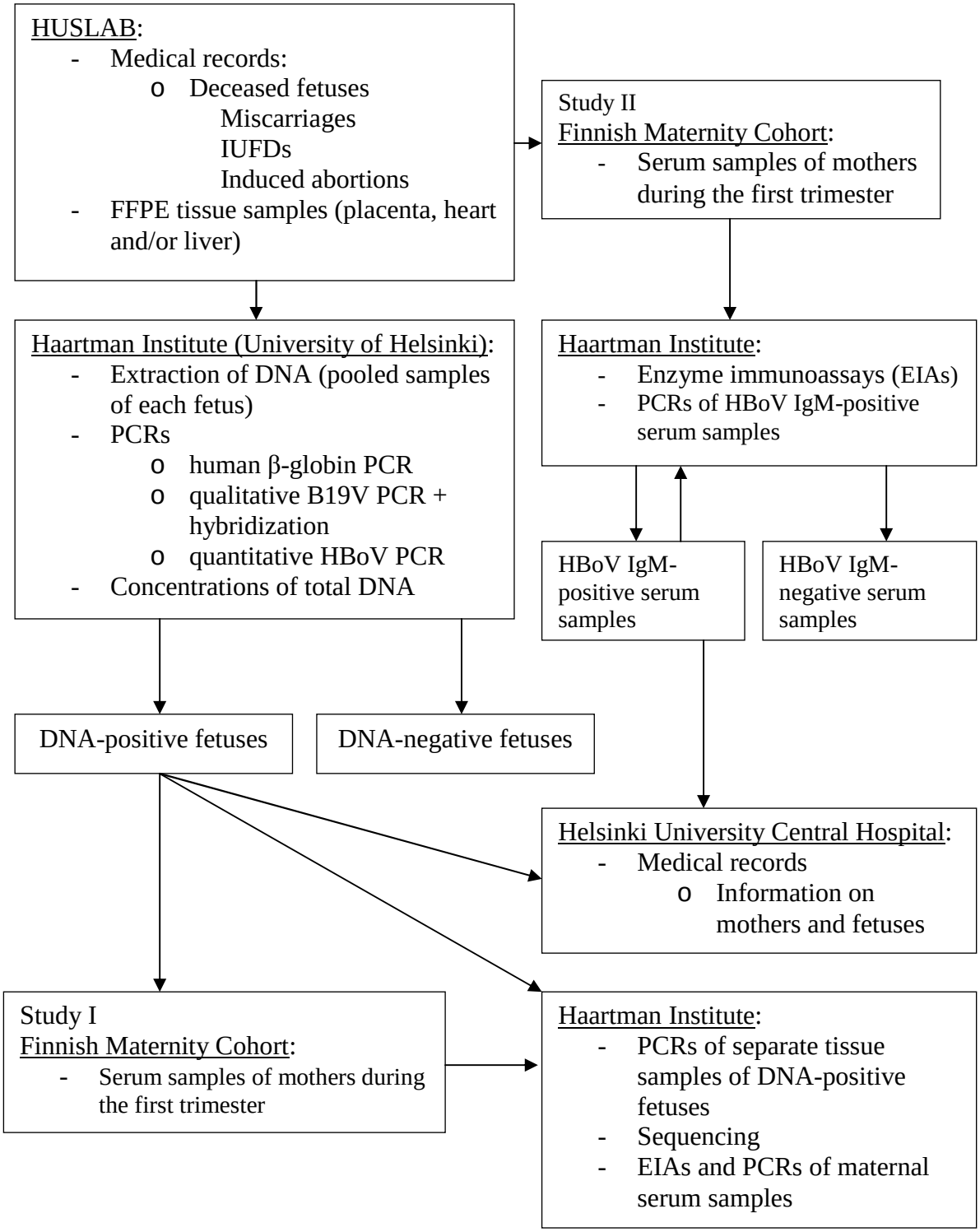
The study consisted of four parts: 1) Detection of B19V DNA (Study I) and 2) HBoV DNA (Study II) from aborted fetuses and IUFDs by PCR; 3) a register-based cohort study of pregnancy outcomes during 1991–2004 among the staff of child-care centers and a comparison group of women from different health care occupations (Study III ); 4) a study on B19V IgG seroconversion among the pregnant child-care employees and the women in the comparison group during a major B19V epidemic in Sept. 1992 to Aug. 1993 (Study IV). The designs of Studies I and II are described in Figure 2, the design of Study III in Figure 3, and the design of Study IV in Figure 4.

This thesis is based on the results of the above-mentioned studies. The study design was approved by the Coordinating Ethics Committee of the Hospital District of Helsinki and Uusimaa. Permission for use of the Finnish Medical Birth Register (MBR), the Register of Congenital Malformations, and medical records was obtained from the Ministry of Social Affairs and Health. Permissions for use of the registers of the Finnish maternity cohort (FMC), the Finnish Centre for Pensions, and Statistics Finland were obtained from the organizations in question. Permission for use of the fetal tissues was obtained from the National Supervisory Authority for Welfare and Health (Valvira).

## 4.2. Clinical samples and data, Studies I and II

The presence of B19V DNA (Study I) and HBoV DNA (Study II) was studied among miscarriages and IUFDs in the Helsinki metropolitan area. Induced abortions performed exclusively for medical reasons served as a control group. Formalin-fixed paraffin-embedded (FFPE) tissue samples of 550 autopsied fetuses were collected during 7/1992–12/1995 and 1/2003–12/2005. The former period was chosen because in 1993 a major B19V epidemic occurred in Finland. The more recent years were selected in order to compare the preservation of DNA in FFPE tissue samples between recent and older material. Human β-globin DNA-negative fetuses were excluded (n=5). Of 10 pairs of twins studied and found to be B19V-DNA negative, only fetus A was included. For the other 22 pairs of twins or multiple pregnancies, only the alphabetically first available fetus was studied (no exclusions). The study finally comprised 535 fetuses: 120 miscarriages, 169 IUFDs, and 246 induced abortions. The time of fetal death ranged from 11–42 gestational weeks. According to official statistical records, a total of 67,858 live births and 290 IUFDs occurred in the Helsinki

Figure 2. Design of B19V and HBoV prevalence studies among miscarriages and IUFDs (Studies I and II).



metropolitan area during the study period. Therefore, 58% (169/290) of the IUFDs in the Helsinki metropolitan area were available for the study. Due to the low proportion of registered miscarriages, their number was small in the study, estimated as <5% of all miscarriages.

In Study I, the serum samples of the B19V DNA-positive mothers from the FMC were tested for B19V antibodies. The FMC – maintained by the Department of Children, Young People and Families of the National Institute for Health and Welfare – includes serum samples from pregnant women (for hepatitis B, *Treponema pallidum*, and HIV screening) normally drawn in connection with the first antenatal visit. All B19V DNA-positive tissue samples were histologically re-examined for signs of viral infection by a pathologist experienced in perinatal pathology. Also, all samples from the B19V epidemic year 1993 were re-tested with a PCR procedure used by Skjöldebrand-Sparre et al. (2000), Tolfvenstam et al. (2001) and Norbeck et al. (2002), to compare the sensitivities of the PCRs. In an additional study, information from medical records was obtained on mothers who were diagnosed to have an acute B19V infection during pregnancy, and were followed up in the Helsinki University Central Hospital during 1998–2007.

In Study II, the HBoV-IgM and IgG antibodies were measured in serum samples of the FMC available from the mothers (n=462; gestational weeks: mean 9, median 9, range 2–36) of the studied fetuses. The mothers' ages ranged from 18 to 45 years (mean 31, median 31). The sera of the mothers with HBoV IgM-positive or borderline results were further examinated by PCR. Also, B19V and Epstein-Barr virus IgM antibodies (Aalto et al. 1998, Kaikkonen et al. 1999) were studied, because acute infections caused by these viruses are known to interfere with IgM assays (Mengeling et al. 1986, Haukenes et al. 1994, Via et al. 2006).

Information was obtained from the pathological records on gestational weeks of all 535 fetuses: gender, hydrops, maceration, chromosomal anomalies or syndromes, intrauterine infections and placental status. In addition, the medical records corresponding to the mothers of the B19V DNA-positive fetuses and HBoV IgM-positive mothers were reviewed for final cause of fetal death, morphological changes, hydrops and possible intrauterine blood transfusions. The information on the mothers included occupation, age, previous deliveries, and infectious diseases during pregnancy.

## 4.3. Data and study populations of the cohort studies

Source population

A register-based cohort study on pregnancy outcomes during 1991–2004 (Study III; Figure 3), and a study on B19V seroconversion during a major epidemic 9/1992–8/1993 (Study IV; Figure 4) were conducted among pregnant child-care employees, including children's nurses and nursery school teachers in Finland. The comparison group – i.e., physiotherapists, pharmacists, opticians, masseuses, rehabilitation nurses, and dental nurses/hygienists – consists of pregnant women working in health care occupations and having a similar socioeconomic status as the child-care employees, but little or no occupational contact with children. The nursery school teachers were identified from the Trade Union of Education and from the Union of Professional Social Workers, while the others were identified from the National Supervisory Authority for Welfare and Health. The source population consisted of 60,926 women born in 1946 or later.

Mothers and pregnancies; the Medical Birth Register (MBR)

Information on the mothers and their pregnancies was obtained from the MBR, founded in 1987. The MBR contains data on the mother's age, deliveries, smoking habits, and socioeconomic status based on maternal occupation; all births and perinatal deaths with data on gestational age, birth weight, and gender are registered.

Study III

Preterm birth, birth weight, SGA (smallness for gestational age), perinatal death and congenital anomalies of the infants of the child-care employees were studied. Altogether 49,501 children were born to 26,934 women during 1991–2004.

SGA was defined as below the 10th percentile birth weight for gestational age in the comparison population. Due to the small number of infants born before gestational week 35, SGA was determined only for infants older than that.

Information on congenital anomalies was obtained from the Register of Congenital Malformations maintained by the National Institute for Health and Welfare. Major congenital anomalies, excluding luxation of hips, retention of testis, and some other minor congenital anomalies, were studied (n=617). Also non-chromosomal anomalies of the cardiovascular, nervous, urinary and musculoskeletal systems, and chromosomal defects were studied separately.

Figure 3. Design of the register-based study on pregnancy outcome among the Finnish child-care employees and the women of the comparison group (Study III).

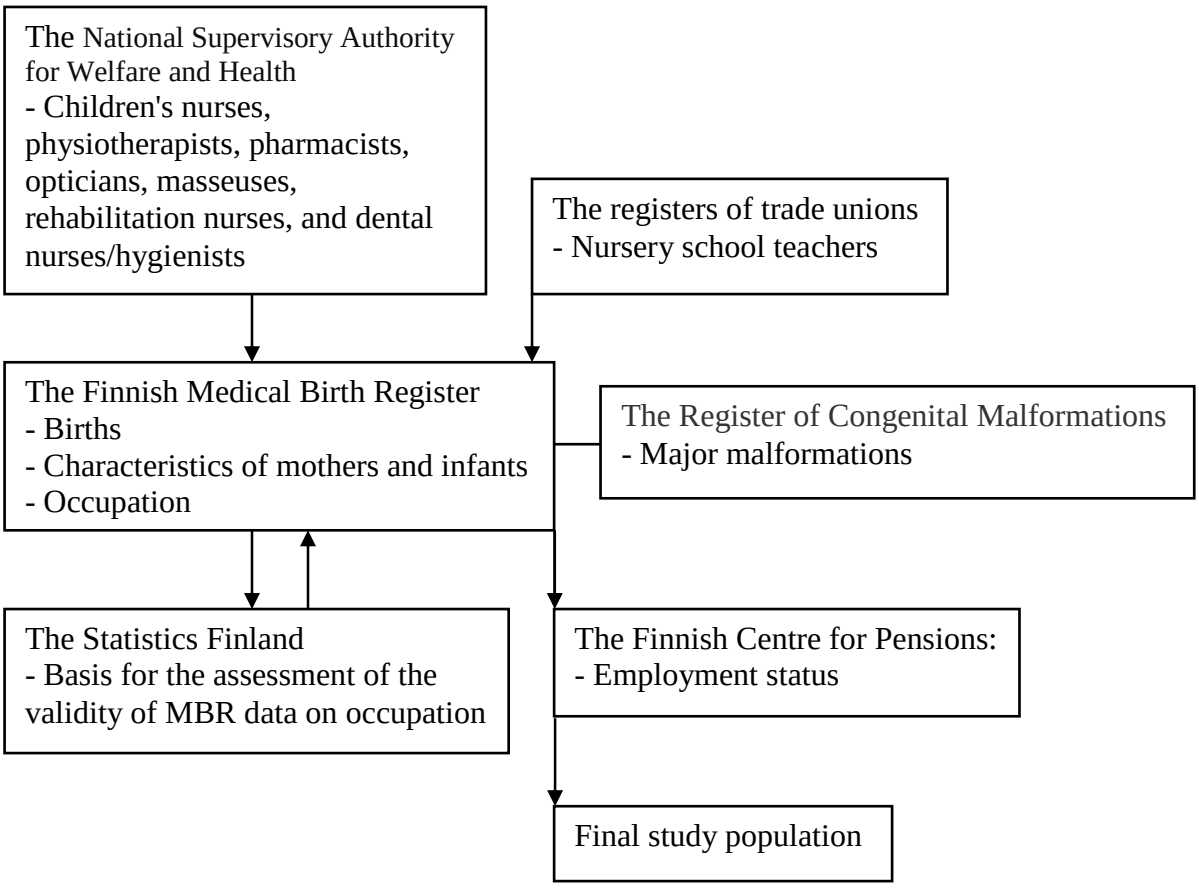
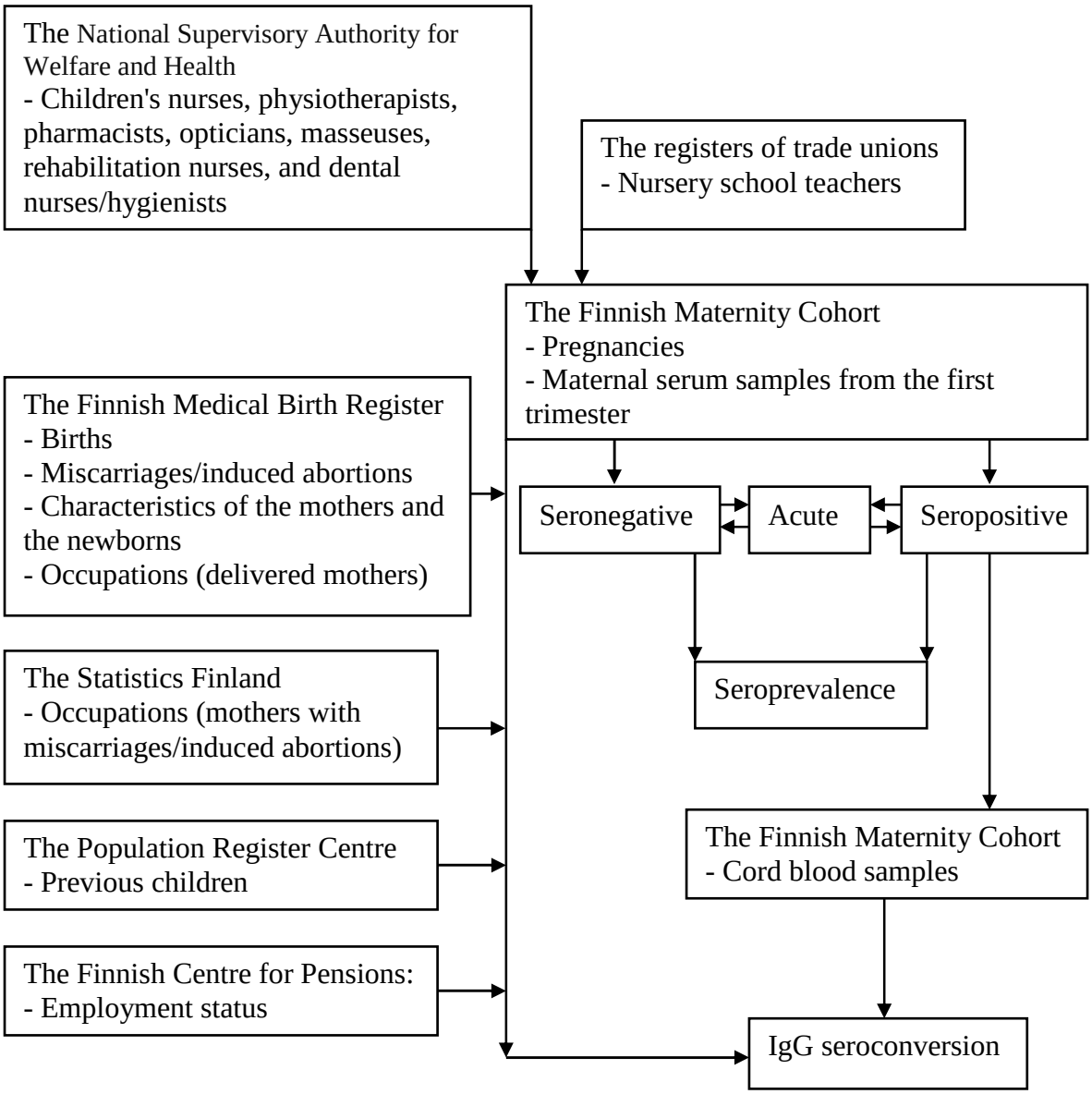


Figure 4. Design of Study IV among the Finnish child-care employees and the women of the comparison group.



Study IV

The data on the source population was first linked with the FMC to identify pregnancies and serum samples around the B19V major epidemic (Figure 4). Information on the sampling date and the expected date of confinement (EDC) were also obtained from the FMC. The mothers' first pregnancies that could have resulted in birth between Sept.1, 1992 – Aug. 31, 1993 were selected (n=4276), because the umbilical cord blood samples (for screening congenital hypothyreosis) were only available for that period. No samples were available for 132 women during the first trimester. Finally, 4144 women were included in the study. Of the pregnancies, 92.0% (n=3812) resulted in birth, 0.3% (n=13) in intrauterine fetal death, and 7.7% (n=319) in miscarriage or induced abortion.

Information on the mothers' older children was recorded in the MBR or in the Population Register Centre. This study was double-blinded: serological definitions and occupational/employment statuses were assessed separately.

Seroprevalence of B19V was examined from the first trimester serum samples (n=4144, mean and median 10 gestational weeks). The cutoff value for a positive IgG was 0.198. A total of 2352 mothers were seropositive, i.e. immune to B19V, and 1792 were seronegative, i.e susceptible to B19V. The effects of the mother's age, years from qualification/joining the trade union, and the number of children were observed.

All the first-trimester serum samples were studied for B19V in-house IgM enzyme immunoassay (EIA). Positive serum samples were further examinated for three IgG-quality tests: VP2-IgG epitope-type specificity (ETS) with two different antigens – a single KYVTGIN peptide (ETS1) and a four-branched KYVTGIN peptide (ETS4) – and VP1-IgG avidity (Söderlund et al. 1995a and 1995b; Kaikkonen et al. 1999). In addition, a commercial IgM EIA (Biotrin, Dublin, Ireland), and PCR (Riipinen et al. 2008) were studied. Acute infections were defined as I) IgG and PCR positive and positive by either in-house or commercial IgM EIA and diagnostic results in two of the three IgG-quality tests, or II) IgG and PCR positive and positive by both IgM EIAs and diagnostic results in one of the three IgG-quality tests, or III) IgG negative, PCR positive and positive by either IgM EIAs. Furthermore, the other samples were defined as a potentially acute B19V infection if they were positive by either PCR or commercial IgM EIA.

B19V seroconversion was studied among the women who were IgG seronegative during the first trimester. Because maternal IgG antibodies are transmitted vertically, IgG seroconversions were detected from the corresponding umbilical cord blood samples (n=819). If seroconversion was detected, comprehensive serologic analyses (ETS1, ETS4, VP1-avidity test, IgM EIAs, PCR) were done. No corresponding cord blood samples were available due to: I) delivery occurred outside the study period; II) miscarriage or induced abortion; III) fetal death or; IV) no samples remained.

Because B19V IgG antibodies begin to appear two weeks after infection (Anderson et al. 1985), IgG seroconversion and also employment follow-up were started two weeks before serum sampling. Correspondingly, seroconversion follow-up ended two weeks before umbilical cord blood sampling. In Finland, maternity leave begins no later than 35 days before EDC. Therefore, follow-up of employment was stopped either seven weeks prior to EDC, or two weeks before the delivery, whichever came first.

Occupation

The occupations of the studied mothers were specified in detail in Studies III and IV. All the studied women represented either child-care employees or health care workers. Furthermore, the MBR was used as the source of the occupation of the mothers whose pregnancies had resulted in birth. Because Study IV included also possible miscarriages and induced abortions (not included MBR registrations), the mother's occupation was determined according to the registered occupation and information from Statistics Finland and/or on the MBR registration of other deliveries.

Employment status

The employment status of the mothers was obtained from the nationwide employment register of the Finnish Centre for Pensions, where all salaried employees are registered.

Study III was restricted to mothers with 60 or more registered employment days during the first 22 gestational weeks (the minimum length of gestation for births and stillbirths) in both studied groups. A 60-day limit was used, because holidays and sick leaves cannot be distinguished from registered information on employment.

In Study IV, the women were divided into four groups according to their occupation and employment status: child-care employees, women in the comparison group, women in other occupations, and unemployed women.

Final study populations

Study III was restricted to singleton births of mothers who were ≥20 years of age at the beginning of their pregnancy. The final study population of Study III included 13,299 and 12,182 births, in the study and the comparison group, respectively.

In Study IV, the women who were ≥ 20 years old and qualified/joined the trade union before pregnancy were included. The final B19V seroconversion population – the mothers with a live birth – included 320 child-care employees, 317 employees in the comparison group, 56 employees from other occupations, and 126 unemployed women.

## 4.4. Laboratory assays

### 4.4.1. Serology (Studies I, II and IV)

B19V IgM EIA (Study IV)

For IgM, a μ-capture format was used. Serum samples diluted 1:200 in PBS and 0.05% Tween (PBST) were applied in duplicate into wells of plates coated with goat anti-human IgM (Cappel/ICN Biomedicals, Costa Mesa, CA, USA) for 60 min at room temperature. After being rinsed five times with PBST, biotinylated B19V VP2 in 0.5% bovine serum albumin (BSA) was applied at a concentration of 10 ng/well and incubated for 45 min at 37ºC. Bound antigen was visualized by using horseradish peroxidase-conjugated streptavidin (Dako, Glostrup, Denmark) at 1:12,000 in PBST plus 0.5% BSA for 45 min at 37ºC, followed by o-phenylenediamine dihydrochloride (OPD; Dako) and $H_2O_2$ for 15 min at 37ºC. The reaction was stopped after 30 min (Nunc polysorp plates) or after 15 min (Costar plates) with 0.5 M $H_2SO_4$. Absorbances at 492 nm were recorded.

B19V IgG EIA (Study IV)

B19V-IgG antibodies were measured by sensitive and specific EIAs employing virus-like VP2 particles as antigen (Kaikkonen et al. 1999). The biotinylated antigens – VP2 capsid at 40 ng/well – in PBS containing 0.05% Tween 20 (PBST) were incubated on streptavidin-coated plates (Labsystems, Helsinki, Finland) for 60 min at room temperature in a rocking (400 rpm) incubator (iEMS, Labsystems). To minimize a nonspecific background, the antigen-sensitized plates were precoated for 3 x 10 min with sample diluents containing a protein and detergent additive (LOY-X-factor, Labsystems). Serum samples 1:200 in PBST were applied, 100 μl/well, in duplicate, for 60 min at room temperature. After being rinsed 3 x 5 min with PBST, anti-human IgG-horseradish peroxidase conjugate (Dako) diluted 1:2000 in the sample diluent was applied. After being rinsed four times with PBST, OPD substrate and $H_2O_2$ were added. The reaction was stopped after 10 min with 0.5 M $H_2SO_4$. Absorbances at 492 nm were recorded.

B19V IgG-avidity EIA (Study IV)

Avidity of B19V IgG antibodies was measured by protein-denaturing EIAs, using the recombinant βVP1 antigen, or VP1u-GST antigen. The antigens were absorbed in PBS into wells (Nunc polysorp) overnight at 22ºC. The wells were washed 2 x 5 min with 8 M urea, 3 x 10 min with PBST, 1 min with sterile water, and 3 x 10 min with LOY-X-factor. The serum samples were diluted serially in PBST in fourfold steps from 1:12.5 to 1:800 or from 1:50 to 1:3200. After 60 min incubation at 37ºC, the wells with the first four dilutions were washed 3 x 5 min with 8 M urea in PBST. The last four dilutions were washed only in PBST. The wells were treated for 60 min with anti-human IgG-horseradish peroxidase conjugate (Dako) diluted 1:2000 in the LOY-X-factor. After being washed 4 x 5 min with PBST, OPD substrate and $H_2O_2$ were added. The reaction was stopped after 30 min with 0.5 M $H_2SO_4$. Absorbances at 492 nm were recorded. Two antibody titration curves (of urea-washed and PBST-washed

wells) were plotted for each sample. End point titers were determined at a cutoff absorbance of A405 = 0.2. IgG avidity was calculated by the ratio of the antibody titers [(urea+/urea-) x 100], and was expressed as a percentage (avidity %). The cutoff point for low avidity was 15%.

B19V IgG-ETS EIA (Study IV)

VP2-IgG epitope-type specificity was studied as described previously (Söderlund et al. 1995a and 1995b; Kaikkonen et al. 1999). VP2-IgG epitope-type specificity was measured similarly to B19V-IgG antibodies, but only the antigens and concentrations were different. According to the items of the biotinylated antigens, VP2 capsid at 40 ng/well or 33.3 ng/well were used. Linear KYVTGIN-peptide (ETS1) or four-branched KYVTGIN peptide (ETS4) were added, 16 ng/well. ETS ratios were calculated by dividing the absorbance of the conformational epitope by the absorbance of the linear KYVTGIN-peptide or the four-branched KYVTGIN peptide. The cutoff point for a low ETS ratio was 10.

Commercial B19V IgM EIA (Biotrin, Dublin, Ireland) (Study IV)

A commercial IgM EIA (Biotrin, Dublin, Ireland) was used to confirm the IgM results.

HBoV IgM and IgG EIA (Study II)

The sera from the mothers were studied by IgM and IgG EIAs as described previously (Söderlund-Venermo et al. 2009). HBoV IgM and IgG EIAs were conducted as described for B19V (described above) except that biotinylated HBoV VP2 VLPs were used at concentrations of 25 ng/well for IgM and 60 ng/well for IgG. IgM-EIA absorbance values of <0.136, 0.136–0.167 and >0.167 were defined as negative, borderline and positive, respectively. Similarly, IgG- EIA absorbance values of <0.154, 0.154–0.188 and >0.188 were defined as negative, borderline and positive, respectively.

## 4.4.2. Extraction of viral DNA

Serum samples (Studies II and IV)

DNA was purified from 50 µl (Study II) and 100 µl (Study IV) of serum with the QIAamp DNA Blood Mini kit (Qiagen). Buffer AL was added to serum with PBS in total 200 µl, and pulse-vortexed for 15 s. After incubating at 56ºC for 10 min, 230 µl ethanol were added and mixed by pulse-vortexing for 15 s. The mixture was applied to a spinn column, and centrifuged at 6000 x g for 1 min. Buffer AW1 was added, and centrifuged at 6000 x g for 1 min. The column was placed in a clean tube, Buffer AW2 was added, and centrifuged at 14,000 rpm for 3 min, and at 8000 rpm for 1 min in a new tube. Finally DNA was eluted in 50 µl of Buffer AE.

Tissue samples (Studies I and II)

All heart, liver and placental tissues available as FFPE blocks were sampled from the blocks as punch biopsies with a volume of 2–4 $mm^3$, and pooled from each fetus. Because many different fetal tissues could be preserved on the same block, this method provided more accurate sampling than ordinary microsection slices. The biopsy instrument was sterilized between each sample by successive treatments with 2% Deconex, water, 0.5 M HCl, water, ethanol, and flaming.

A FFPE fetal and a B19V DNA-positive FFPE placental tissue samples were used to select and optimize the deparaffination and DNA-extraction methods. Several deparaffination and DNA-extraction methods, including the classical xylene-ethanol and phenol-chloroform extraction (Coombs et al. 1999), were examined. The salting-out method, which was modified from previous descriptions, was chosen, because it was easy, economical, safe, and of course most efficient.

The tissue-containing paraffin layer was cut off from each sample with a sterile knife. Viral DNA of tissue pools were purified by proteinase K digestion for 48 h at +48ºC with proteinase K replenishing at 24 h. This was followed by a salting-out extraction. Tissue lysates were boiled for 10 min at +95ºC and centrifuged for 5 min at 13,200 rpm at +4ºC. The solution under the paraffin layer was transferred into a new tube. NaCl was added to a final concentration of 1.2 M, and the sample was mixed for 20 s and then re-centrifuged for 5 min at 13,200 rpm at +4ºC. The DNA in the supernatant was transferred into a new tube, precipitated with absolute ethanol overnight and centrifuged for 30 min at 13,200 rpm at +4ºC. Ethanol was removed and the DNA at the bottom of the tube was washed carefully with ice-cold 75% ethanol. Finally, DNA was redissolved in 60 µl of $H_2O$. The DNA solution was stored at -20ºC until used. Water, as negative control, was inserted between every 20 samples and prepared along with the tissue pools.

Total DNA concentrations of all the IUFD extracts were measured using a spectrophotometer (Nanodrop Technologies). The total DNA concentration was >100 ng/µl (mean 882 ng/µl; median 802 ng/µl) in >99% of the FFPE tissue extracts.

### 4.4.3. PCR procedures (Studies I, II and IV)

Precautions in PCR

Because of the high sensitivity of PCR, strict precautions were taken to avoid false-positive results in all PCR studies. Separate rooms for sample preparation, DNA amplification and detection, aerosol-resistant pipette tips and disposable racks were used to avoid DNA carry-over. B19V genotype-1 plasmid DNA was included as a positive control in each B19V PCR set. Water was used as a negative control between every 20 samples in all PCR procedures, as in DNA extraction.

The human β-globin gene was amplified from each pool for PCR inhibition and DNA stability control. A 109-bp fraqment was amplified by the forward primer PCO3 (5'-ACACAACTGTGTTCACTAGC-3') and the reverse primer PCO4 (5'-CAACTTCATCCACGTTCACC-3'). In a 1:10 dilution, 1 µl of the extract was used per PCR reaction. In addition, a total of 25 µl of reaction mixture consisted of 10 x GeneAmp PCR buffer I (Applied Biosystems, CA, USA), 200 µM of each deoxyribonucleotide triphosphate (dNTP), 0.5 µM of each primer and 1.25 U AmpliTaq Gold (Applied Biosystems, CA, USA). The PCR reaction consisted of an initial step at 95ºC for 10 min, followed by 40 cycles at 95ºC for 1 min, 58ºC for 1 min, and 72ºC for 1 min, and a final step at 72ºC for 5 min.

B19V PCR (Studies I and IV)

As the DNA in archival tissues can be deteriorated, PCR amplicons were designed to be short, but still capable of detecting all three B19V genotypes. FFPE fetal tissue and B19V DNA-positive FFPE placental tissue were used to select and optimize the subsequent PCR. A 248-bp fragment of the VP1 gene was amplified by the forward primer 9f (5'-TGACTTTAGGTATAGCCAACTGG-3') and the reverse primer 3r (5'-CTTCTGCAGAATTAACTGAAGTC-3'). The analytical specificity and sensitivity were tested with 10-fold dilution series (both in water and in FFPE-DNA extracts) of plasmids containing B19V genotypes 1, 2 or 3 (GeneBank AY504945, AY044266 and AY083234, respectively) and of the genomic DNA extracted from the B19V-positive FFPE placenta.

To minimize the effects of PCR-inhibiting factors, 3 µl (1:10 dilution) of the extract was used per PCR reaction. In addition, a total of 25 µl of reaction mixture consisted of 10 x GeneAmp PCR buffer I (Applied Biosystems, CA, USA), 200 µM of each dNTP, 0.5 µM of each primer (9f and 3r) and 1.25 U of AmpliTaq Gold (Applied Biosystems, CA, USA). The PCR reaction consisted of an initial step at 95ºC for 10 min, followed by 40 cycles at 95ºC for 30 s, 52ºC for 30 s, and 72ºC for 30 s, and a final step at 72ºC for 5 min.

Nested B19V PCR (Study I)

Purified DNA from the same DNA extracts from the epidemic year 1993 was re-amplified by the nested PCR used by the Swedish group (Broliden et al. 1998, Tolfvenstam et al. 2001, Norbeck et al. 2002). In a nested PCR designed to amplify a 284-bp fragment of the NS1 gene 2 µl of a 1:10 dilution of the extract was used as the template. First, a 369-bp fragment was amplified by the forward primer NSf1 (5'-GGCAGCATGTGTTAAAGTGG-3'), and the reverse primer NSr2 (5'-CAGTTGTTGTAGTGTTCCC-3'). The first amplification consisted of 35 cycles at 94ºC for 30 s, 55ºC for 45 s, and 72ºC for 1 min. In the nested round, a 285-bp fragment was amplified by the forward primer NSf2 (5'-AATACACTGTGGTTTTATGGGCCG-3') and the reverse primer NSr2 (5'-CCATTGCTGGTTATAACCACAGGT-3'). The second amplification consisted of 25 cycles at 94ºC for 30 s, 55ºC for 45 s, and 72ºC for 1 min.

HBoV qPCR (Study II)

DNA was purified from 100 µl of serum with the QIAamp DNA Blood Mini kit (Qiagen) and eluted in 50 µl. DNA samples were screened from two pools with 2.5 µl each per reaction, in a total volume of 25 µl. The TaqMan universal PCR master mix (PE Applied Biosystems, CA, USA) was used, and the HBoV PCR was performed according to Allander et al. (2005) with some modifications (Kantola et al. 2008). The reaction mix consisted of 1 x GeneAmp PCR buffer II (100 mM Tris HCl, pH 8.3/500 mM KCl) (Applied Biosystems), 2.5 mM $MgCl_2$, each dNTP at 0.2 mM, 20 pmol each of forward primer 188F (GACCTCTGTAAGTACTATTAC) and the reverse primer 542R (CTCTGTGTTGACTGAATACAG), and 2.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, CA, USA). The thermal cycler was Mx3005P QPCR System (Stratagene), and the settings were 95°C for 10 min, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. A quantifiable result was obtained with as few as 10 copies/µl of the HBoV Stockholm 2-containing plasmid (St2, GenBank no. DQ000496).

### 4.4.4. B19V southern hybridization (Studies I and IV)

The PCR results in Studies I and IV were confirmed by Southern hybridization with a digoxigenin-labeled probe (nt 3081-3328 of B19V NAN, GenBank AY504945) designed to recognize all three B19V genotypes. The probe was prepared by incorporating digoxigenin-11-deoxyuridine triphosphate (DIG-11-dUTP) (Roche Diagnostics GmbH, Mannheim, Germany) during PCR with primers 9f and 3r and using the B19V genotype-1 plasmid (GeneBank AY504945) as the target sequence. The constituent tissues of the B19V-positive tissue pools were re-examined individually.

Because only single-stranded DNA can be hybridized, DNA on an agarose gel was denatured by 1.5 M NaCl and 0.4 M NaOH for 45 min, and after that depurinated with 3 M NaCl and 80 mM NaOH for 15 min. Denatured DNA was transferred to Hybond N+ nylon filter by capillary action overnight, neutralized by PBS, and dried for 15 min at 80ºC; this also cross-linked the DNA to the membrane.

Pre-hybridization was performed to block non-specific sites. The filter was soaked in prehybridization solution for 30 min at 42ºC, then in hybridization solution overnight at 42ºC. Thereafter it was washed two times in SSC and in 0.1% SDS for 2 x 5 min at room temperature, and in 0.1% SSC and 0.1% SDS for 2 x 15 min at 68ºC. The filter was rinsed in buffer 1 (0.1 M Tris-HCL, 0.15 M NaCl, pH 7.5), and incubated in buffer 2 (1 x TBS, pH 8.0, 0.05% Tween, 10% blocking buffer; Sigma Aldrich, MO, USA, and 20.5 g sucrose/100ml) for 30 min. Anti-digoxigenin-alkaline phosphate (anti-DIG-AP) conjugate (Roche Diagnostics GmbH, Mannheim, Germany) was diluted at 1:5000 in buffer 2, and the filter was incubated in this solution for 30 min, washed in buffer 1 for 2 x 15 min, and balanced by buffer 3 (0.1 M tris-HCL, 0.1 M NaCl, 0.05 M $MgCl_2$, pH 9.5). Finally, the filter was

incubated in the dark for 1–3 h with substrates including 45 µl of 5-bromine-4-chlorine-3-indolylphosphate, and 35 µl of Nitro blue tetrazolium chloride (Roche Diagnostics GmbH, Mannheim, Germany) in 10 ml of buffer 3.

## 4.5. Statistical methods

In Study III, marginal models were used to examine the associations between work and pre-term birth, perinatal death, SGA, and congenital anomalies. The pregnancies of the same woman were assumed to correlate with each other and therefore the generalized estimating equations method with logit link function and exchangeable correlation structure were selected to assess the risks. Odds ratios (OR) with confidence intervals are shown in the paper. In the analysis of birth weight, the full-term births were analyzed as described by Wilcox (2001), and linear regression was used. As potential confounders, maternal age at the beginning of pregnancy, smoking habits, socioeconomic status (SES), previous deliveries, gender (not in SGA analyses), and calendar period of the child's birth were included in all the multivariable models. Birth weight was also adjusted for gestational age. The analyses without adjustment were repeated to account for the potential role of gestational age at birth as an intermediate variable. Hospital districts were adjusted for in the analyses of congenital anomalies. The used models accounted for dependence between births by the same woman.

In Study III, three sets of sensitivity analysis were conducted to account for potential validity problems in the data. 1) The main analysis was repeated after excluding physiotherapists and masseuses on the one hand, and dental nurses/hygienists on the other. Physiotherapists and/or masseuses are exposed to short waves, microwaves, ultrasound, and physical exertion, which are related to adverse pregnancy outcome (Feychting et al. 2005), as are mercury and nitrous oxide in dental work (Lindbohm et al. 2000). 2) The data were analyzed by two calendar periods, because after the educational reform, the proportion of child-care employees fell from 55% (1991–1997) to 48% (1998–2004). In addition, 3) the primiparous women were analyzed separately, because mothers of under three year-old children are entitled to a child-care leave, which can not be discerned from the registered employment information.

In Study IV, different statistical methods were tested, e.g. Poisson regression, but the proportional hazard regression (HR) was found to be the most applicable to estimate the relative risk of B19V seroconversion between the study and the comparison groups. Logistic regression was used to estimate ORs of an acute B19V infection for the child-care employees as compared to the women in the comparison group. In the analysis of acute B19V infections, the study period was split into seven equal sub-periods, and adjusted for in the multivariable model to visualize infection peaks within the study period. In addition, the number of own children aged 3–10 years was adjusted for; the selected age range was used because no excess

risk of B19V infection was found among the mothers with under three or over ten year-old children. In the analysis of seroconversion, the number of children and Helsinki metropolitan area were adjusted for. Additional analyses were conducted excluding women with children under three years of age, because they are entitled to child-care leave. The age of the women was not adjusted for, due to the strong correlation with the number of children.

The statistical analyses were carried out using the statistical software package SAS (version 9.1, SAS Institute Inc., Cary, NC, USA), procedure GENMOD for generalized estimation of equations and procedure MIXED for regression.

# 5. RESULTS

## 5.1. Parvovirus B19 infection in deceased fetuses

Five B19V DNA-positive fetuses were found, all of them representing genotype 1. Of the IUFDs 2.4% (4/169), and of the miscarriages 0.8% (1/120) were B19V-DNA positive. None of the induced abortions were positive. The fetal deaths occurred during all trimesters. B19V DNA was detected in five of five placenta, three of four myocardial tissue samples, and one of four hepatic tissue samples.

Four of five B19V DNA-positive fetuses, representing all trimesters, were hydropic. The only non-hydropic fetus died at gestational week 39. No other infections or anomalies were detected in the B19V DNA-positive fetuses. B19V infection was assumed to be the cause of death of three fetuses, and placental abruption the cause of death of the other two. Histologically, no inclusion bodies were found, but three fetuses had large early erythroblasts, indicating fetal anemia. Two of them had been treated with intrauterine transfusions.

All mothers of the five B19V DNA-positive fetuses had serologically confirmed acute or recent B19V infection before the fetal death; four of the five mothers were tested at gestational weeks 6–14, and one mother at week 22. According to the medical records, two of the mothers had symptoms typical of acute B19V infection (rash or arthralgia) three and nine weeks before fetal death. Four mothers had children aged 2–9 years, and one worked with children as the director of a child-care center.

During the epidemic year 1993, 5.1% (3/59) of the fetuses were B19V-DNA positive: 4.9% (2/41) were IUFDs and 5.6% (1/18) were miscarriages. During non-epidemic years 0.9% (2/230) of the fetuses were B19V-DNA positive: 1.6% (2/128) were IUFDs, whereas none (0/102) was a miscarriage.

During the years 1998–2007 in the Helsinki University Central Hospital, 17 pregnant women were diagnosed with an acute B19V infection, and were followed by ultrasound for up to 12 weeks after B19V infection. Five of them overlapped with the study period 2003–2005. Three of these had symptoms typical of B19V infection; two were anemic and received intrauterine transfusion, but only one survived; one fetus had cardiac insuffiency and ascites without anemia, and survived. The other 14 fetuses had no clinical evidence of acute B19V infection, and all of them survived. Of the B19V-infected mothers, 35% (6/17) worked with children as nursery school teachers, school teachers or nurses in a pediatric hospital.

## 5.2. Human bocavirus infection in deceased fetuses

None of the autopsied fetuses were HBoV-DNA positive. Of the 462 mothers, ten (2.2%) were HBoV-IgG negative, and four (0.9%) had a borderline IgG result. The other 448 (97%) mothers were IgG positive, four (0.9%) with a positive, and three (0.6%) with a borderline HBoV-IgM result. The sera of these seven mothers were HBoV-DNA negative during the first trimester. Of the four HBoV IgM-positive mothers, one displayed histological evidence of an acute unspecific intrauterine infection, one had a serologically suspected listeria infection without histological evidence of intrauterine infection, and one had a urinary tract infection at the time of fetal death at gestational week 35. Only the latter mother had a high HBoV IgM absorbance value, whereas the other three mothers were barely positive during the first trimester. None of these four mothers had serological evidence of acute B19V or EBV infection, previous deliveries, nor did they work with children.

## 5.3. Pregnancy outcomes among child-care employees

No associations were found between child-care work and preterm birth, perinatal death or SGA. Furthermore, the results of the sensitivity analyses of the first births, two calendar periods and subsets, excluding selected occupations, were similar between the studied groups. During the B19V epidemic year (1993), seven perinatal deaths were found among the child-care employees and five in the comparison group. However, the numbers were too small for meaningful analysis.

The adjusted mean birth weight of the infants of the child-care employees was slightly higher than that of the infants born to the comparison group (14 g, 95% CI -1–29). Among primiparous women the difference in adjusted mean birth weight was reduced to 6 g (95% CI -12–25). No essential changes were observed in the analyses without adjustment for gestational age (mean difference in all births 15 g, 95% CI -1–31). As anticipated, the infants of smoking women had a lower mean birth weight (3517 g) than the infants of non-smoking women (3629 g), as had the infants of primiparous (3525 g) compared to multiparous women (3730 g), and correspondingly, girls (3554 g) compared to boys (3695 g).

No essential differences were found between the infants of the child-care employees and the comparison group regarding all congenital anomalies (adjusted OR 1.10; 95% CI 0.92–1.32) of chromosomal, cardiovascular, nervous, or musculoskeletal systems. However, the infants of the child-care employees born in the latter calendar period had a slightly increased risk of congenital anomalies (OR 1.25; 95% CI 0.96–1.61), especially urinary tract defects (OR 2.11; 95% CI 1.09–4.09).

The child-care employees had more previous deliveries, especially during the former calendar period, and they were more likely to be upper white-collar workers than the women in the comparison group. There were only slight or no differences in the mothers' ages, smoking habits, or gender of the infants between the study and comparison groups.

## 5.4. Parvovirus B19 IgG seroconversion among pregnant child-care employees

The child-care employees were more likely to be B9V-seropositive (n=1269, 59%) than the women in the comparison group (n=1083, 54%) ($p<0.05$). Seropositivity expectedly increased with age, the number of own living children, and in the case of the child-care employees with years from qualification/joining the trade union. Only slight or no differences were found between the studied groups in regard to the women's ages, number of children, children's ages, or years from qualification/joining the trade union.

During the first trimester, 17 mothers had a definitely acute infection and 15 others had a potentially acute infection. Due to the small numbers, these two groups were combined for statistical analyses. The child-care employees had probably a higher risk of acute B19V infection than the women in the comparison group (adjusted OR 2.18; 95% CI 0.87–5.45). The risk increased along with the number of children aged 3–10 years. Epidemic fluctuation occurred during the study period.

The maternal seroconversion rates were 6.6% (21/320) among the child-care employees and 3.8% (12/317) among the women in the comparison group (HR 2.49; 95% CI 1.20–5.17). The corresponding annual seroconversion rates were 12% and 7%. The risk of the child-care employees increased if the mothers with children under three years of age were excluded (HR 5.77; 95% CI 1.75–18.99). The risk increased along with the number of children. The women from the Helsinki metropolitan area had a lower risk of having acute B19V infection than the women living in other parts of Finland (HR 0.44; 95% CI 0.19–1.01).

Of the corresponding cord blood samples of the seroconverted mothers, 20% (9/44) were B19V-IgM positive, and 34% (15/44) were B19V-DNA positive.

# 6. DISCUSSION

## 6.1. Parvovirus B19 infection in deceased fetuses

During 1992–1995 and 2003–2005, low prevalences of B19V DNA were detected in the Helsinki metropolitan area among fetuses from IUFDs (2.4%) and miscarriages (0.8%). Even during a major B19V epidemic in 1993, the prevalence of B19V DNA among IUFDs was lower (4.9%), than that (14–15%) reported in some previous studies (Tolfvenstam et al. 2001, Norbeck et al. 2002).

In Study I, most of the B19V DNA-positive IUFDs complied with the typical pattern of B19V infection, i.e., viral infection in the second trimester, hydrops, nucleated erythroblast-containing tissues, and serological evidence of an acute or recent maternal B19V infection. In two previous studies (Tolfvenstam et al. 2001, Norbeck et al. 2002), most of the DNA-positive fetuses had no hydrops (17/20) and they were deceased during the third trimester (16/20). Only three mothers had evidence of an acute or recent B19V infection; their two fetuses (gestational weeks 22 and 25) were the only cases with histopathological evidence of B19V infection. All in all, in these studies only few fetuses complied with the typical pattern of B19V etiology. Thus, there are probably fewer B19V-associated fetal deaths than reported. This view is supported by Peterson et al. (2004), who detected a low B19V DNA occurrence (4%) among IUFDs. It is noteworthy that the frequencies of hydropic B19V DNA-positive IUFDs (1.8%), and the mothers with an acute B19V infection (2.4%) in Study 1 are in line with the frequencies observed in previous studies (1.1–2.2% and 1.1–4.3%, respectively; Tolfvenstam et al. 2001, Norbeck et al. 2002).

Skjöldebrand-Sparre et al. (2000) studied only placental tissue samples of IUFDs during the third trimester, and found 7.5% (7/93) of them to be B19V-DNA positive. Placental tissue always contains both maternal and fetal tissues, which can not be separated. The finding could therefore point to both maternal and/or fetal acute or recent B19V infection, i.e. viremia, or persistence of B19V in placental tissues. Of the fetuses of B19V-infected mothers 25–50% are believed to be infected. This is supported by Skjöldebrand-Sparre et al. who found that one third of the fetuses (1/3) of all the available fetal tissue samples of B19V DNA-positive cases (3/7) were B19V-DNA positive. This result is based on a small number of cases, however. On the other hand, in Study I, during third trimester pools containing placental tissue samples with or without fetal tissue samples, 2.5% (2/81) were B19V-DNA positive. In both of two B19V DNA-positive cases, also the fetal tissues were positive, indicating intrauterine fetal infection. All in all, the numbers of the B19V DNA-positive fetuses in the study of Skjöldebrand-Sparre et al. (2000) are relative to the results of Study I.

The low prevalences of B19V-related IUFDs are supported by surveillance studies of B19V-infected pregnant mothers (Miller et al. 1998, Enders et al. 2004); almost all fetal deaths occurred during the first half of pregnancy. In Study I, the prevalence of B19V-related miscarriage is not reliable, because only a few per cent of all miscarriages could be included. Thus, no conclusions on B19V-related miscarriages could be drawn based on this study.

B19V have been shown to have life-long tissue persistence after infection (Söderlund et al. 1997, Norja et al. 2006). This probably holds true also for fetal tissues. The B19V transmission rate from mother to fetus is 25–50%, whereas a 5–10% excess risk of fetal loss has been related to maternal B19V infection. Most of the B19V-infected fetuses will therefore recover from the infection. However, the fetus can die also of other causes, and B19V could thus be an innocent bystander, or predispose to fetal death. This could partly explain the reported third-trimester IUFDs without a typical pattern of B19V infection in the Swedish studies. Also in Study I, the only nonhydropic IUFD had, at gestational week 39, B19V DNA-positive heart tissue but no other signs of B19V infection; the mother of this fetus had evidence of acute B19V infection at the beginning of her pregnancy.

Compared to the non-epidemic years, a 6-fold excess in the frequency of B19V DNA-positive fetuses was observed during a major epidemic in 1993. Although the results are based on small numbers of cases, they comply with those of previous studies, in which the risk of having B19V infection was approximately 10-fold higher during epidemics than during nonepidemic periods (Valeur-Jensen et al. 1999, Jensen et al. 2000). Of note: in some other studies no evident differences were found in the number of B19V-associated IUFDs between epidemics and nonepidemic periods (Skjöldebrand-Sparre et al. 2000, Norbeck et al. 2001).

## 6.2. Absence of HBoV DNA in deceased fetuses

All 535 fetal/placental tissue sample pools were HBoV-DNA negative, suggesting that these fetuses did not have an acute HBoV infection at the time of death. In line with this finding, Enders et al. (2009) detected no HBoV DNA in amniotic fluid from 87 fetuses with hydrops, anemia or isolated effusions. However, they did not study fetal tissues.

Approximately 97% of the mothers of the studied fetuses were HBoV-IgG positive, and most probably immune to HBoV. These findings are in line with those of another study in which all 19 pregnant women with fetal hydrops and 94% (47/50) of pregnant women with normal ultrasound findings were HBoV-IgG positive (Enders et al. 2009). HBoV IgG has also been detected in >94% of healthy adults (Endo et al. 2007, Lindner et al. 2008b, Söderlund-Venermo et al. 2009).

None of the four HBoV IgM-positive mothers had viremia at the time of blood sampling, suggesting absence of ongoing HBoV infection at the beginning of pregnancy. This finding does not totally exclude the possibility that the mothers, and also the fetuses, could have been HBoV-infected during pregnancy, because the viremic period is short in HBoV infections (Söderlund-Venermo et al. 2009). Only one of the four HBoV IgM-positive mothers had a high HBoV IgM absorbance value; the fetal death occurred at gestational week 35, seven months after infection. Therefore, the fetal death was probably not caused by acute HBoV infection.

Although the other pathogenic human parvovirus, B19V, can lead to fetal death by causing fetal anemia or carditis, HBoV is not known to cause anemia or heart diseases. Only in one study was HBoV DNA detected in cardiac tissue samples, indicating possible infection and/or persistence of HBoV in cardiac tissue (Kuethe et al. 2009). The animal bocaviruses CnMV and BPV can cause transplacental infections and adverse pregnancy outcomes. Embryonic resorption and fetal death usually occur during the first two trimesters, whereas during the third trimester the fetus is protected by the developed immune system. In Study II, no evidence of transplacental HBoV infection was found. High seroprevalence among adults points to immunity, but additional studies are needed to determine the possibility of reinfection, viral persistence or reactivation of HBoV.

## 6.3. Pregnancy outcomes among child-care employees

The risk of adverse pregnancy outcome was determined at the population level among child-care employees during 1991–2004. Little or no differences were found in the occurrence of preterm birth, perinatal death, SGA and congenital anomalies between the infants of the child-care employees and those of the comparison group. The infants of the child-care employees had a slightly higher mean birth weight than the infants of the comparison group, but the difference disappeared in the subset of the first births.

In Study III, the findings on perinatal death, preterm birth and SGA are in accordance with the results of Canadian studies (McDonald et al. 1987, 1988a and 1988b). However, neither an increased risk of major anomalies nor low birth weight were found among the infants of the child-care employees, although they were detected in the Canadian studies (McDonald et al. 1987, Armstrong et al. 1989). The occurrence of preterm birth, perinatal death and congenital anomalies was similar to that among all Finnish newborns (Gissler et al. 2009).

In the sensitivity analysis, the data were restricted according to parity, mother’s occupation, and the year of the child's birth. The only essential finding was a slightly increased risk of urinary tract defects among infants of child-care employees born in the latter calendar period.

During the study period of 1991–2004, the coverage of our data on some occupations decreased, while the coverage of data on registered congenital anomalies increased. However, it is unlikely that these trends could have an effect on the risk of urinary tract defects. The explanation for these findings remains unknown.

The strength of Study III was the access to nationwide data containing about 13,000 singleton births, both of the child-care employees and of the women in the comparison group during 14 years; this was the largest study on the pregnancy outcomes of child-care employees. Contrary to Study 1, in the Canadian studies the numbers of pregnancies of the child-care workers were small, and the comparison group consisted of women from 60 different occupations.

Only slight or no differences were found in perinatal death or congenital anomalies between the study and the comparison groups. Child-care employees are at an increased risk for various virus infections that can lead to fetal death (B19V) (Miller et al. 1998, Ender et al. 2004), or congenital anomalies (CMV, VZV, influenza) (Edwards et al. 1995, Orney et al. 2006). About a half of all child-care employees are susceptible to CMV and B19V infections. The annual seroconversion rates of these mothers have ranged from 8–20% for CMV (Pass et al. 1990, Murph et al. 1991). For B19V, the annual seroconversion rates have generally varied from 1–13%, but could be as high as three-fold among child-care employees (Valeur-Jensen et al. 1999). At most, half of the CMV or B19V infections are vertically transmitted, and most of the infected fetuses survive without anomalies. Adverse pregnancy outcomes due to these infections are threfore rare at the population level in child-care work, making it difficult to observe excess risks. This holds true also for the other, infrequent reproductive hazards – i.e., heavy physical exertion, noise, and psychosocial strain – in child-care work.

## 6.4. Parvovirus B19 seroconversion during an epidemic

The seroprevalences of the child-care employees (59%) and of the women in the comparison group (54%) are in line with a recent Finnish study in which 58% of pregnant women were seropositive (Alanen et al. 2005). Similar results have been reported in many population-based studies (Valeur-Jensen et al. 1999, Jensen et al. 2000, van Gessel et al. 2006, Mossong et al. 2008). In Study IV, IgG seroprevalence increased with years from qualification/joining the trade union. Accordingly, Gilbert et al. (2005) found an association between increasing B19V IgG seroprevalence and years of work in child-care centers.

An over two-fold increased risk of B19V infection was found among the child-care emloyees compared to the women of the comparison group in this cohort study. In a population-based Danish study, women with a kindergarten teacher’s education had a three-fold risk of acute

B19V infection as compared to women with other educational backgrounds (Valeur-Jensen et al. 1999). Contrary to Study IV, the mother's actual occupation and work status were not specified in either the study or the comparison groups.

The annual seroconversion rates were 12% among the child-care employees and 7% among the comparison group women during the B19V epidemic. Three previous studies on seroconversion focussed on the population level. The annual seroconversion rates in the Danish population were 13% during epidemics and 1.5% during non-epidemic periods (Valeur-Jensen et al. 1999). Because the risk of the child-care employees was three-fold, even up to 40% of them could be infected during an epidemic. This calculation is nevertheless based on small numbers of B19V-infected child-care employees. In another Danish study, the annual B19V infection rate was 13% (Jensen et al. 2000), but the B19V IgM-seropositive mothers during their first antenatal visit were included in the calculations of infection rate. The women working with children were not at an increased risk of infection. In a Dutch study, the seroconversion rate during pregnancy was 2.4% (van Gessel et al. 2006) during mixed epidemic and non-epidemic times.

The child-care employees were at a greater risk of having an acute or recent B19V infection than the women in the comparison group during the first trimester. As anticipated, the risk increased with growing number of children. In the Helsinki metropolitan area, the risk of seroconversion was lower than in other parts of Finland. The reason for this could be based on the lower number of older children or on local epidemics.

Of the seroconverted mothers, 20% had an IgM-positive and/or 34% had a B19V DNA positive corresponding cord blood sample, indicating intrauterine fetal infection. In some previous studies, 25–50% of maternal B19V infections have been transferred to the fetus (PHLS 1990, Koch et al. 1998). However, our result is an underestimate, because fetuses that are infected early in pregnancy may not appear IgM positive later on, or they were not able to produce antibodies due to an undeveloped immune system. Also, no cord blood samples were available from the succumbed fetuses.

## 6.5. Validity issues

### 6.5.1. Registers

Several data registers were used in Studies III and IV. The source populations consisted of women who were either qualified for the selected occupations or members of selected trade unions; the former group included all studied occupational groups other than nursery school teachers. In Finland, according to the Trade Union of Education, over 95% of nursery school teachers belong to the union. The National Supervisory Authority for Welfare and Health contains information on all qualified health care employees. Therefore, the coverage of the

data on the source population was sufficient. However, after an educational reform in August 1995, the coverage of data on children's nurses, dental nurses and rehabilitation nurses began to fall (Study III) when these occupations were included under a new non-specific title of practical nurse, and thus could no longer be identified from the register.

The Medical Birth Register (MBR), which has been in existence since 1987, covers almost 100% of the births in Finland, and the information on birth outcomes is reliable (Gissler et al. 1995).

The MBR was used as the source register to obtain the most recent information on the mothers' occupations during pregnancy. Because information on occupation is registered sometimes during the first pregnancy (M. Gissler, personal communication), the validity of the information on occupation from the MBR was tested by comparing it with that obtained from Statistics Finland at two time points: the last weeks of 1995, and of 2000. The information on occupations in the MBR was in agreement with that of Statistics Finland in 83% of the child-care employees, and in 87% of the women in the comparison group. Of the mismatched pairs 7% could not be found in the data of Statistics Finland, probably due to unrecognized child-care leaves or the short, one week, reference period at the end of the year. Therefore, the validity of the information on occupation from the MBR is probably better than might be concluded according to this comparison.

Information on congenital anomalies was obtained from the Register of Congenital Malformations, which has received data on congenital anomalies from hospitals, health-care professionals and cytogenetic laboratories. Data have also been drawn from the MBR, the Care Register, the Register of Induced abortions, the Register of Visual Impairment, the National Supervisory Authority for Welfare and Health, and the Cause of Death Statistics. The data content and data collection practices were revised in 1993, and thereafter the data coverage has been regarded as good. According to EUROCAT (http://www.eurocat-network.eu/), the anomalies were divided into major and minor ones. Only major congenital anomalies were included in this study.

The employment statuses of the mothers' during pregnancies were obtained from the nationwide employment register of the Finnish Centre for Pensions, where all salaried employees are registered. To avoid the possibility that the mothers' holidays or sick leaves could cover the entire employment period, only mothers who were employed ≥60 days during the first 22 weeks of gestation were included (Study III). Pregnancy outcomes were also examined among primipara to avoid potential misclassification caused by unrecognized child-care leaves (Study III). The results were similar, indicating that this problem apparently had no impact on the study.

### 6.5.2. Missing data

In Studies I and II, 58% of all IUFDs that occurred in the Helsinki metropolitan area during the study period were investigated. The proportion was similar to that observed by Norbeck et al. (2002). Data were missed, because not all IUFDs were autopsied, e.g. because the parents did not grant consent. Also, all histological glasses or FFPE blocks could not be found. However, the missing data were not associated with B19V or HBoV infection, and thus did not cause sampling bias. Only an estimated <5% of all miscarriages were available for Studies I and II, because most miscarriages occur without the woman being aware of her pregnancy, and, in general, the registration rate of miscarriages is low. Because only a few percent of the miscarriages could be included, sampling bias is very probable. Therefore, veritable prevalence rates of B19V DNA could not be detected among the miscarriages.

In Study III, many confounding factors were adjusted for, but unfortunately, no information was available on the mothers' body mass index (BMI), diseases, use of alcohol and marital status. Mothers with diabetes and/or obesity are known to have heavier infants than healthy mothers (Boney et al. 2005). Maternal BMI and hypertension tend to have an effect on SGA and preterm birth (Zeitlin et al. 2001). In addition, unmarried status has been associated with higher perinatal mortality (Seidman et al. 1990, Forssas et al. 1999). The study groups and the comparison groups consisted of women who worked in the social and health care sector, and were believed to be relatively homogeneous groups regarding their life style, such as smoking habits, as observed in this study. However, about 75% of the child-care personnel were upper white-collar employees, while only 35% of the women in the comparison group belonged to this group. Adjustment of SES may have partly reduced the confounding factors involving living habits.

In Study IV, a large number of serum samples from the first trimester and cord blood samples were studied during a major B19V epidemic in Finland. Unfortunately, some cord blood samples were missed mostly because they were used in other studies, and information on them was not available.

In Study IV, a few B19V IgG seroconversions were probably missed, because cord blood samples were not available from the succumbed fetuses. Thirteen of the fetuses died. Miscarriage or induced abortion were suspected among the pregnant mothers on whom there was no information in the MBR (n=319). B19V infection is known to cause fetal death typically during the first half of pregnancy (PHLS 1990, Miller et al. 1998, Enders et al. 2004), but rarely later than that (Enders et al. 2004). Some miscarriages could therefore have been associated with B19V infection. B19V is not considered to be teratogenic, and thus induced abortions have no effect on the seroconversion rate.

### 6.5.3. Laboratory assays

In Study IV, seroconversion was defined as an increase of IgG, but various laboratory tests – IgM, VP1-IgG avidity, VP2-IgG epitope-type specificity (ETS1 and ETS4), commercial IgM EIA (Biotrin, Dublin, Ireland), and PCR – were used to verify acute or recent B19V infections.

Highly sensitive PCRs were used to detect DNA in Studies I, II, and IV. Because the DNA in archival tissue samples can be deteriorated, PCR amplicons were designed to be short yet capable of detecting all three B19V genotypes. Because of the highly sensitive PCR, strict precautions were taken to avoid contamination. In order to avoid DNA carry-over, separate rooms were used for sample preparation, DNA amplification and detection, and also aerosol-resistant pipette tips and disposable racks were used. Water served as a negative control in DNA extraction and in all PCR procedures.

The differences between the results of Study I and the Swedish studies (Tolfvenstam et al. 2001, Norbeck et al. 2002) may well be due to the various preservation methods of tissue samples or different technical procedures. As in Study I, Norbeck et al. (2002) used FFPE tissue samples from the period 1993–1997. In Study I, lower total DNA concentrations were found among the IUFDs of the former study period than among those of the latter period, indicating fatiguing DNA in long-term storage. Nevertheless, B19V DNA-positivity was also found in samples with low DNA content. Tolfvenstam et al. (2001) used freshly frozen tissue samples, as did Skjöldebrand-Sparre et al. (2000), but the latter also re-examined all freshly frozen B19V DNA-positive placental samples as FFPE, and found only slight differences in the results.

Because formalin fixation is known to decrease PCR sensitivity due to deterioration of DNA, various methods were explored for deparaffination and DNA preparation. The classic xylene-based method, which has been shown to decrease the detection sensitivity of PCR, and was used by Norbeck et al. (2002), was also tested. Paraffin-melting and salting-out procedures (Laitinen et al. 1994, Rivero et al. 2006) resulted in the best detection sensitivity. Furthermore, to discover possible differences in sensitivity of PCRs between these studies, all samples from the epidemic year 1993 were re-examined with the nested PCR of the Swedish studies (Broliden et al. 1998). Positive results were obtained for only two of the three fetuses that were positive in the assay used initially in Study I; this difference is probably due to the shorter amplicon of Study I PCR. However, these two PCR assays were equally sensitive in a dilution series of a B19V-containing plasmid. All in all, in >99% of IUFD extracts, the human β-globin gene could be amplified, and the total DNA concentrations were >100 ng/µl (mean 882 ng/µl, median 802 ng/µl) implicating good DNA preservation and lack of PCR inhibition.

## 6.6. Parvovirus B19 as an occupational risk

The spread of infectious diseases is common in child-care centers, because of the close personal contacts and poor hygiene of young children. Child-care employees are exposed to various infections by touching, diapering, washing and feeding the children, as well as by touching environmental surfaces and via the air.

The annual B19V seroconversion rate was over two-fold among the child-care employees, compared with the comparison group. The risk of the child-care employees increased, if the mothers of children under three years of age were excluded. Similar to a previous study (Gillespie et al. 1990) the child-care employees were more often B19V-seropositive than the women in the comparison group. An association between increasing B19V seroprevalence and age of the mothers or years from the qualification/joining the trade union were found among the child-care employees, but not among those in the comparison group. These findings are in accordance with a Canadian study (Gilbert et al. 2005), and support the concept that child-care employees are at an increased risk of B19V infection.

At the population level, an increased risk of B19V infection has been observed among child-care employees (Cartter et al. 1991, Valeur-Jensen et al. 1999, Jensen et al. 2000). However, the risk has been described to be much greater during a community B19V epidemic in a school and a hospital. Tuckerman et al. (1986) described a B19V epidemic in a primary school where almost all of the B19V-susceptible teachers were infected. Furthermore, Bell et al. (1989) reported 36% and 38% attack rates among B19V-susceptible hospital staff who had been in contact with the B19V-infected patients.

All in all, during the first half of pregnancy and B19V epidemics the B19V-related fetal loss rate for child-care employees with unknown immunologic status is 1–2/1000. This result served as a base for the following calculations: 1) approximately 40% of fertile-aged child-care employees are B19V-IgG seronegative, and thus susceptible to infection; 2) about 7% of these women are assumed to be B19V-infected during 29 weeks (mean follow-up time in Study IV); and 3) B19V-infected mothers have up to 10% excess risk of having a miscarriage during the first half of their pregnancy (gestational weeks 5–20) (1000 * 0.4 * 0.07 * 0.1 * (15/29) = 1.4). Respectively, the B19V-related fetal loss rate for seronegative child-care employees is 4/1000. However, during very early pregnancy B19V-related fetal loss rate (subclinical miscarriages) is unknown, and therefore the risk is probably more.

## 6.7. Management of parvovirus B19 infection during pregnancy

B19V infection can cause adverse pregnancy outcomes leading to fetal anemia and fetal death. If a pregnant woman has been in close contact with a person suffering from a non-vesicular rash illness, the immunity of the woman should be tested for B19V (Figure 5).

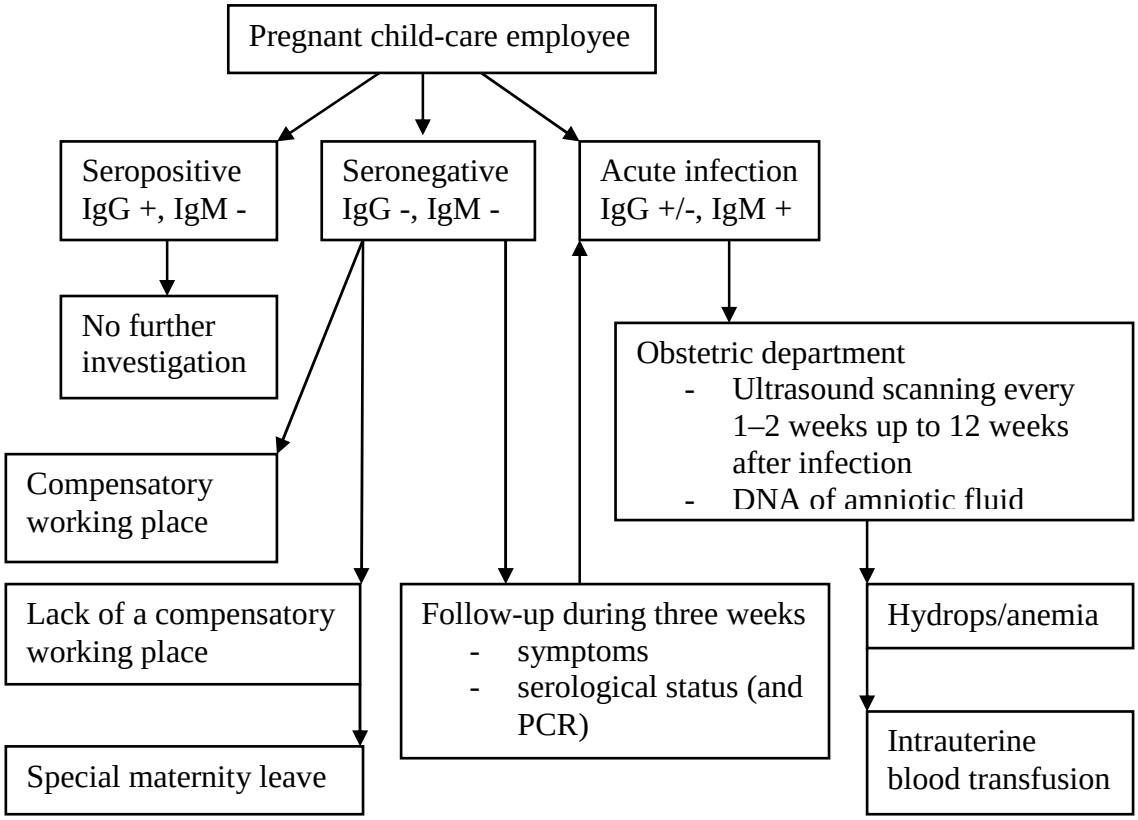
1) If the woman is B19V-IgG positive and IgM negative and of past immunity, she is immune, and no further investigations are needed.

2) If the woman is B19V-IgG negative and IgM negative, she is not immune. In order to rule out an acute infection, the possible appearance of antibodies and/or symptoms should be followed for three weeks. The most reliable diagnostics for primary B19V infection during pregnancy comprise comprehensive serology complemented with PCR of maternal serum.

3) If the woman is B19V IgG negative/positive and IgM positive, she should be sent to an obstetric department to be followed by ultrasound scanning every 1–2 weeks up to 12 weeks after infection. If the fetus develops anemia and hydrops, intrauterine blood transfusion can be performed.

Study IV verified that the personnel working in child-care centers are at an increased risk of contracting B19V infection. When employees begin the work, they should be informed about the occupational hazards, including the reproductive risk factors. The B19V serological status of persons who work with young children should be determined. Also in maternity clinics, pregnant women need to be warned about the consequences of infection and the importance of avoiding exposure whenever possible. Information on management during epidemic should be available from maternity clinics and occupational healths. The best means of preventing infectious diseases is thorough hand-washing. Sometimes even more stringent procedures are needed to avoid contagion. During B19V epidemics, the preventive procedure of choice for nonimmune pregnant employees is a transfer to another workplace. Child-care centers are typically municipal workplaces, and a possible transfer to another workplace could be managed. Problems may appear for instance in private sector child-care centers. If a substitute workplace cannot be arranged, a special maternity leave should be granted, especially if infection occurs during the first 20 gestational weeks.

Figure 5. B19V epidemic in a child-care centre and management of pregnant child-care employees.



# 7. CONCLUSIONS

A low B19V-DNA prevalence was detected in the fetuses from the IUFDs. Most of the B19V DNA-positive fetuses were hydropic with evidence of acute or recent maternal B19V infection.

No HBoV DNA was detected in the fetal tissues or the placental samples. The seroprevalence of HBoV was high among the pregnant women indicating that most of them were immune to HBoV.

At the population level, child-care employees are not at an increased risk of adverse pregnancy outcomes, i.e., preterm birth, perinatal death, SGA, or congenital anomalies. These findings do not exclude the possibility of incidental occupational risks, e.g. infections, which may be hazardous to pregnancy.

Child-care employees are at an increased risk of contracting B19V infection, especially during epidemics.

Infectious diseases are very common among pre-school children, and some of these infections may be harmful to pregnancy. Furthermore, pregnant women undergo immunological changes which are necessary for the maintenance of pregnancy, but which make them more susceptible to infections. Because the risk of B19V infection and the ensuing risk of fetal loss are increased among child-care employees, preventive procedures, such as a change of workplace or a special maternity leave, are essential for nonimmune pregnant women during B19V epidemics.

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