The lack of routine surveillance of Parvovirus B19 infection in pregnancy prevents an accurate understanding of this regular cause of fetal loss and the risks posed by occupational exposure

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In Europe, fetal loss due to Parvovirus B19 (B19V) is under-reported and a poorly addressed occupational risk to pregnant women. This is exemplified internationally, where it was unmentioned in the last two European Centre for Disease Prevention and Control (ECDC) annual surveillance reports or its 2009 special report on infections in pregnancy. To assess this potential for underestimating B19V fetal loss in pregnancy, we undertook a systematic review of practice in Northern Ireland in the management and reporting of B19V infections over a 12-month period of heightened transmission, one of six observed in a span of 9 years. Pregnant and non-pregnant women presented with symptomatic infection in 24 and 93 % of confirmed B19V infections, respectively, with no difference in viral loads. There was underinvestigation of viral causes of fetal loss, with only 143/2739 (5 %) tested for B19V, and a failure to follow up most non-immune women tested following rash contact. Occupational exposure was recorded in 31/60 (51.6 %) of pregnancies audited following rash exposure, the majority teachers or day care workers. Against a background seroprevalence of 66.5 % immunity in women of child-bearing years, two patterns of infection were identified. Firstly, pregnant women investigated for a rash or exposure to slapped cheek syndrome, where an infection incidence of 18 % was observed, resulted in 42 confirmed infections, all proceeding to healthy term deliveries. Secondly, pregnant women with unsuspected infection had six cases of confirmed B19V fetal loss, including four of 22 (18 %) diagnosed at autopsy, of which three were non-hydropic. While many studies have reported B19V fetal loss in pregnancy, there are no robust public health surveillance figures to draw on. That all six confirmed fetal losses came from the small number of miscarriages/stillbirths investigated, 143 out of 2739, suggests inadequate follow-up of those pregnancies where B19V-related fetal loss may be most common, and supports the need for enhanced surveillance pilots to address this significant gap in public health knowledge.

INTRODUCTION

Infectious causes of fetal loss are poorly addressed. In the UK in 2007, infection was recorded as a cause of stillbirth in 3.5 % of cases, with the majority, up to 72 %, remaining unexplained, and less than half having a standard or coroner’s post-mortem (CEMACH, 2007). In obstetric practice up to 12–15 % of clinically recognized pregnancies miscarry, with the likelihood of losing a viable pregnancy falling to 3 % after 8 weeks gestation and to 1 % after 16 weeks gestation (Simpson, 1990). Recurrent miscarriage is the spontaneous loss of three or more consecutive pregnancies with the same biological father and affects 1–2 % of couples (Duckitt & Qureshi, 2011), with 25–50 % of women experiencing one or more sporadic miscarriages. Most women who suffer up to three first trimester miscarriages will have a successful pregnancy (Duckitt & Qureshi, 2011), but some will require referral and investigation for a definitive cause (Regan et al., 2011). Viral infection is not regarded as a cause of recurrent miscarriage and determining its role in sporadic fetal loss is inconsistent.

Parvovirus B19 (B19V) fetal loss results from infection of erythroid precursor cells and profound fetal anaemia; late gestational loss, possibly linked to myocardial involvement,
Parvovirus B19 diagnosis in pregnancy

has also been speculated (Skjøldebrand-Sparre et al., 2000). National seroprevalence varies by country, thought in part to be due to different childcare arrangements. In England, approximately 40% of women of child-bearing age are non-immune to B19V, with no systematic seroprevalence data for the rest of the UK or Republic of Ireland (Mossong et al., 2008). While it is a recognized cause of fetal loss, the true extent is still controversial, with studies reflecting both relatively rare fetal involvement (4.2% loss) (Enders et al., 2010) or relatively high fetal involvement (16% fetal loss) (Beigi et al., 2008). Relying on research studies for gauging the actual level of loss is no substitute for regular gathering and analysis of accurate routine data at a community level. This is not happening for two reasons. The first concerns the follow-up of fetal loss for an infectious aetiology, where obstetric practice focuses on recurrent miscarriage, which is regarded as having a non-infectious basis (Regan et al., 2011). The second concerns poor surveillance for B19V infection, epitomized by its absence from the last two European Centre for Disease Prevention and Control annual communicable diseases surveillance reports (ECDC, 2011; ECDC, 2009a) and from its 2009 special report on infections in pregnancy (ECDC, 2009b). Locally in Northern Ireland there are no public health records kept of B19V-associated fetal losses.

However, accurate information is crucial for public health guidance, infection control and providing the rationale for vaccine development. Currently, identification of B19V infection in pregnancy relies on poorly directed communication between healthcare professionals, including midwives, obstetricians, general practitioners, virologists, pathologists and public health physicians. The mostly asymptomatic nature of B19V infection in pregnancy, its absence from both antenatal and occupational screening, and the lack of a requirement for notification impede both professional vigilance and proper recording of its role in fetal loss. Major B19V outbreaks are described as running in a 4–6 year cycle (Oliveira et al., 2002; Nicolay & Cotter, 2009), but increasing awareness and testing of pregnant women in relation to slapped cheek syndrome has resulted in more focused testing in this patient sector and a trend to recognition of more frequent cycles of transmission.

Northern Ireland has a population of 1.7 million people and has approximately 25 000 annual live births per annum, managed by 12 obstetric units in five separate healthcare trusts. Diagnostic virology is delivered through a regional virus service in Belfast, ensuring complete access to the viral investigation of acute infections and miscarriages in the region. In one of six periods of heightened B19V transmission noted over a 9-year span, a proactive approach was taken to follow B19V exposure and infection against the background of standard obstetric practice. No additional samples were requested and no additional tests were undertaken outside of the standard laboratory protocols. Rather, a more proactive approach was taken to offering advice to healthcare staff managing the pregnancy and to following up on its outcome. The principal aim was to determine the impact of B19V infection in terms of B19V-associated fetal loss, where testing could be improved, and the appropriateness of clinical management following the exposure of pregnant women to B19V infection.

METHODS

Confirmation of B19V infection. B19V IgG and IgM were tested using commercial assays from Biotrin International (Dublin) and followed the manufacturer’s instructions. Patients screening positive for B19V IgM were further tested for B19V DNA using a validated quantitative real-time PCR (qRT-PCR) assay targeting the NS1 gene of the virus. The qRT-PCR has a dynamic range of 12 logs. Antenatal booking bloods retained in line with national standards (UK National Screening Committee, 2010) were retrieved, where possible, when a B19V infection had been confirmed in pregnancy. During the 12-month period, 26 booking bloods were retrieved for B19V IgG analysis to check for seroconversion or for infection at the time of the booking appointment using additional tests for B19V IgM and DNA; this was in line with the laboratory policy to retrieve, where available, a booking blood when B19V was confirmed in pregnancy for evidence of infection at the time of the booking or for subsequent seroconversion. B19V infection during pregnancy was accepted as confirmed where the following criteria were met: both B19V IgM and DNA were detected in maternal blood; maternal B19V IgG seroconversion was confirmed; fetal tissue at autopsy was positive for B19V DNA and this was supported by the histopathology findings following review with the paediatric pathologist.

Seroprevalence. To determine the background immunity of females in the main child-bearing years at the time of exposure to a B19V-like rash, the B19V IgG antibody status of females aged 15–39 years and previously tested was reviewed over an 11-year and 2-month period from January 2000 to February 2012 inclusive. The sera of women with a confirmed acute B19V infection or who seroconverted following exposure were excluded. In total, results were available for 3921 women.

Seasonal transmission of B19V and testing of male and female patients. Using a single positive B19V IgM test as evidence of an acute infection, the monthly numbers of confirmed cases were plotted between January 2002 and December 2011 to determine any seasonal patterns of transmission. In addition, the number of B19V IgM requests on sera from male and female patients aged 15–44 years were also determined for the 3-year period before and after the issuing of a circular on B19V infections in pregnancy by the Public Health Agency (PHA). The circular, HSS (MD) 11/200, was issued in March 2008, and alerted staff in general practices and obstetric units to the need for appropriate vigilance concerning B19V infections during pregnancy.

B19V requests for maternal infection or exposure. Over the 12-month period specimens for B19V testing were received from 211 general practice surgeries and 21 hospitals; requests were received from throughout Northern Ireland and reflected the local population densities. A total of 2082 B19V-specific IgM tests were performed on bloods from 1846 patients (1622 female, 224 male). Of those tested, 681 came from pregnant women following exposure to potential B19V infection, usually slapped cheek syndrome, or because of a suspected clinical infection. As part of enhanced reporting during this period, virology staff contacted the requesting physician to discuss newly diagnosed infections in women who had been identified as pregnant on the request form or whose age was in the child-bearing years. Following a locally agreed algorithm, advice was given

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concerning obstetric referral where a B19V infection was confirmed. For public health reporting purposes, pregnancy outcome was also recorded. The presence of rash and arthralgia symptoms was recorded where available for pregnant and non-pregnant women with confirmed B19V infection.

**B19V requests for fetal ultrasound anomaly, miscarriage or stillbirth.** Using the International Standard Diagnostic Classification (ICD10) records over the 12-month period there were 2620 miscarriages and 119 stillbirths. From these, 194 bloods and 22 autopsy specimens were received for investigation of miscarriage and stillbirth. In addition 25 specimens were received and tested for B19V following confirmation of an in utero anomaly, mainly hydrops fetalis, during routine obstetric ultrasound. The clinical details provided used a number of minimalistic request formats including: request for TORCH (toxoplasmosis, other infections, rubella, cytomegalovirus and herpes simplex virus) serology; request for TORCH with B19V serology; in utero death; stillbirth; fetal hydrops; miscarriage, including recurrent miscarriage. B19V IgM testing was undertaken for 143 patients, and where positive, a test for B19V DNA was also undertaken; B19V tests were not routinely undertaken for recurrent miscarriage during the period reviewed. Referred autopsy tissue was tested for B19V DNA.

**Follow-up in general practice of non-immune pregnant women exposed to B19V infection and determination of risk factors for exposure.** A total of 147 pregnant women were tested following exposure to B19V and found seronegative for parvovirus B19V IgM and IgG. The post exposure management of these women was reviewed and audited against Health Protection Agency (HPA) guidelines, which advise a further test after 4 weeks where the patient is non-immune (HPA Rash Guidance Working Group, 2011). For patients who did not have a follow-up blood, the general practitioner was contacted in an attempt to establish the reasons behind the lack of serological follow-up. Potential risk factors, including occupation, for acquiring B19V infection were sought (Watt et al., 2011).

**Data analysis.** The influence on gender bias of the PHA advisory circular on B19V infections in pregnancy and the presence or absence of symptoms in pregnant and non-pregnant women with confirmed B19V infections was compared using the chi-square statistic; values with P<0.05 were regarded as significant. Epi Info Version 3.5.3 (Centers for Disease Control and Prevention, USA) was used for statistical analysis and patient demographics.

## RESULTS

### Seroprevalence

Of those females tested, 1316/3921 (33.5 %) were non-immune to B19V. The ages of those tested showed a normal distribution, with a mean age of 30 years for both immune and non-immune patients. The non-immune frequency ranged from 46.1 % for 15–19 year olds to 28.7 % for 35–39-year-old females, respectively (Table 1). There was an increase in seroprevalence of B19V infection of 17.4 % in women in the main child-bearing years.

### Seasonal transmission of B19V and testing of male and female patients

Between January 2003 and December 2011 there were a total of 500 confirmed cases of B19V recorded (Fig. 1). Over this 9-year period there were six obvious periods of enhanced transmission peaking in the spring or summer of each respective year, with a further minor period in 2011. The number of sera tested from male and female patients aged between 15 and 44 years of age for the 3-year period before and after the release of the advisory PHA circular showed a clear impact of the circular, principally on enhanced testing of females. Before the circular, 1683 female and 203 male sera from the targeted age range were tested for B19V IgM over the 36-month period, while afterwards, 3620 female and 181 male sera were tested ($X^2=72, P<0.001$).

### Maternal B19V infection confirmed without fetal involvement

Of the 1846 patients tested, 138 screened positive for B19V IgM, of which 42 pregnant and non-pregnant women were diagnosed. The respective median ages of pregnant and non-pregnant women were 31 years (range 16–44) and 37 years (range 17–50), and requests for women in the pregnant group came equally from general practice and antenatal clinics, mostly following exposure to slapped cheek syndrome. Presence or absence of symptoms was available for 82 women. Pregnant women were noted to have a symptomatic infection in only 10/42 (24 %) cases compared with 37/40 (93 %) of non-pregnant women ($X^2=39.5, P<0.001$), with arthralgia a presenting symptom in 4/42 and 31/40 cases, respectively. Accepting that 66.3 % of the 681 women tested were already immune to B19V, this represents a rate of infection of 18 % following exposure of a non-immune woman to a B19V infection during this period of heightened transmission. All patients in whom B19V IgM was detected were positive for B19V DNA, with respective median serum loads in pregnant and non-pregnant women of $1.1 \times 10^5$ ($1.8 \times 10^3–1.3 \times 10^{12}$ copies ml$^{-1}$) and $1.8 \times 10^5$ ($5.6 \times 10^3–5.9 \times 10^6$ copies ml$^{-1}$). One woman presented at

<table>
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<th>Age range (years)</th>
<th>No. tested</th>
<th>No. antibody-negative</th>
<th>Proportion non-immune</th>
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<tr>
<td>15–19</td>
<td>165</td>
<td>76</td>
<td>46.1 %</td>
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<tr>
<td>20–24</td>
<td>532</td>
<td>191</td>
<td>35.9 %</td>
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<td>1096</td>
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<td>1357</td>
<td>442</td>
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<tr>
<td>35–39</td>
<td>771</td>
<td>221</td>
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34 weeks gestation following contact with slapped cheek syndrome and was B19V antibody-negative but had a B19V DNA load $1.37 \times 10^{12}$ copies ml$^{-1}$. All of the pregnant women with confirmed B19V were referred for routine fetal monitoring and all had uneventful pregnancies and healthy term babies.

**Maternal B19V infection confirmed with fetal involvement**

There were six fetal losses confirmed in eight pregnancies with evidence of fetal involvement, including fetal hydrops, miscarriage and stillbirth. Three of 25 women tested because of hydrops found on routine ultrasound, at 20, 24 and 27 weeks gestation, had B19V confirmed, with one fetal loss. Over the same period, 194 women were investigated for a viral cause of miscarriage at a median gestational age for fetal loss of 25 weeks (range 8–41 weeks). Of these, 143 were tested for B19V involvement and 2/143 (1.4%) were positive for B19V IgM and DNA, with respective B19V loads of $1.8 \times 10^{5}$ and $7.3 \times 10^{9}$ copies ml$^{-1}$. In these two cases, miscarriage took place at 17 and 21 weeks, respectively. One of these had a fetal autopsy specimen co-tested, which confirmed B19V infection with a viral load of $1.7 \times 10^{10}$ copies ml$^{-1}$. Two other maternal bloods that tested B19V IgM-negative were from pregnancies where fetal tissues were also co-tested and which had viral B19V loads of $1.62 \times 10^{10}$ and $3.64 \times 10^{10}$ copies ml$^{-1}$, respectively. Specimens from a further fetal autopsy had a load of $1.5 \times 10^{10}$ copies g$^{-1}$, but in that case no maternal blood was co-tested. In the 4/22 (18%) fetal tissues tested and confirmed with B19V, one, at 21 weeks gestation, was hydropic, while the others, at 14, 17 and 22 weeks gestation, were not hydropic.

**B19V seroconversion**

Of the 42 women with B19V infection confirmed by detection of IgM and DNA at clinical presentation, a booking blood was retrieved for 26; in 13 cases a booking blood was not requested and in three cases the infection took place before a booking blood was taken. Seroconversion was observed in 19 women. In seven pregnancies, seroconversion was not observed, as the booking blood was already seropositive for IgG and IgM in five cases and IgM alone in two cases. For these cases the stage of pregnancy ranged from 9 to 17 weeks gestation. Where seroconversion was demonstrated, 17/19 were confirmed in pregnancies over 20 weeks gestation. Fetal loss was observed in 1/19 patients who seroconverted and 1/7 where seroconversion could not be demonstrated.

**Follow-up and occupation of non-immune pregnant women exposed to B19V infection**

Of 147 pregnant women who tested negative for B19V-specific IgM and IgG on their first general practitioner visit following exposure, 57 (38.8%) had follow-up serological testing performed at a mean of 28.7 days between specimens. Of these, 3/57 (5.2%) seroconverted between blood samples, confirming acute B19V infection. The gestational ages were 15, 21 and 27 weeks, and all had healthy term babies after follow-up fetal monitoring. The remaining 90 (61.2%) did not have follow-up bloods tested for B19V, and details were audited for 60 of these women. The commonest reason given, in 39/60 (65%) cases, for non-follow-up was non-development of symptoms of B19V in the post-exposure period, while miscarriage was the second commonest factor identified in 6/60 (10%) pregnancies. Other explanations for lack of follow-up included: pregnancy greater than 20 weeks gestation; the patient gave birth within 4 weeks of the initial test; the patient was no longer a patient at the surgery; the patient did not attend a follow-up appointment as advised; the rash-in-contact was confirmed not to be parvovirus. Twenty-seven (45%) women had documented exposure to a child within their household, either their own child or that of a relative, and 31/60 (51.6%) had contact with B19V via their occupation, the majority of whom were teachers or day care workers.

**DISCUSSION**

Increasingly in Northern Ireland, pregnant women are tested for B19V immunity following exposure to slapped cheek syndrome, and the increase in seroprevalence of 17.4% observed over the child-bearing years in this review confirms the force of infection over this age range and the increased risk that this period poses for pregnant women. Since B19V is not offered as part of antenatal or occupational screening,
this could reflect a growing public awareness, probably web-based. Alternatively, the PHA and Chief Medical Officer (CMO) circulars could have encouraged more proactive testing of women, where previously inappropriate reassurance would have been given (Mitchell, 2009; Doherty, 2008). Increased testing has resulted in a growing confirmation of B19V infections in pregnant women, and the 12-month period described in this manuscript was one of six heightened community transmission periods recorded between 2002 and 2011 (Fig. 1). During this 12-month period, B19V infections were proactively followed to improve the effectiveness of confirming a diagnosis of B19V infection in pregnancy, confirming its role in fetal loss, and to identify deficiencies at both clinic and laboratory levels that could indicate issues to be addressed.

The overall infection rate of B19V in those tested following exposure or because of rash was 18 %, with a 5 % seroconversion rate observed in non-immune women retested following exposure. There was a clear difference in the reason for testing in pregnant and non-pregnant women, with the former in the main being tested because of exposure to a rash and while asymptomatic, and the latter because of an actual clinical infection, usually rash with arthralgia. However when addressed through follow-up discussions with the women’s general practitioners, it was clear that pregnant women more often had completely asymptomatic infections. While the majority of non-pregnant women of child-bearing age presented with a rash and arthralgia, 76 % of pregnant women with confirmed infection remained asymptomatic. Awareness that B19V is frequently asymptomatic during pregnancy is important for healthcare workers managing these patients, as was underlined by a lack of follow-up of the majority of non-immune women after significant exposure. A failure to confirm infection will prevent appropriate referral for fetal monitoring. Compounding this was the lack of follow-up for those non-immune women who miscarried. While an educational initiative showed improvement in a reaudit for follow-up of asymptomatic non-immune women, there was no improvement in the investigation of miscarriages, which remained at approximately 10 %, and lends support to the lack of investigation of fetal loss for an infectious cause (Watt et al., 2011).

Only 194 out of 2060 women with fetal loss had serum submitted for a viral screen, and of these only 143 were tested for B19V involvement. A small number underwent fetal autopsy and were co-tested for B19V. Five fetal losses were confirmed over the 12-month period, contrasting with eight reported over an overlapping 11-year period in the Republic of Ireland, again reflective of the lack of appropriate public health records for B19V-related losses in pregnancy (Nicolay & Cotter, 2009). The failure to test all 194 blood specimens for B19V IgM and the small number of booking bloods retrieved for serotesting showed laboratory inconsistency, partly reflecting the need for retrieval of booking bloods from another laboratory, a problem now corrected by storing these in the virology laboratory. Test requests were often inadequate, with a continuing over-reliance on the TORCH mnemonic, which has been discouraged since 2001 (Regan et al., 2011). Maternal B19V IgM could not be relied on for confirming infection and additionally would need testing of (a) the maternal blood at fetal loss plus the booking blood for B19V IgG; (b) the booking blood for B19V DNA; (c) fetal tissue, if available, for B19V DNA. The deficiencies of the current system that could underestimate B19V fetal loss are: an underinvestigation of fetal loss for a viral aetiology; inconsistent laboratory protocols for testing for B19V in fetal loss; the inappropriateness of relying on a maternal B19V IgM result; in the absence of fetal material the need for testing the maternal booking blood for B19V seroconversion or IgM and DNA as appropriate; the inconsistency of sending both maternal and fetal specimens for analysis.

The median gestation age at loss was 25 weeks. Autopsy confirmed B19V infection in 5/6 fetal losses, with the sixth loss occurring shortly after diagnosis of hydrops. Fetal loss is thought commoner when infection takes place before 20 weeks gestation (Miller et al., 1998; Enders et al., 2010), and was observed in 1/19 (5 %) pregnancies where seroconversion was demonstrable and in 1/7 (14 %) where B19V antibody was already present at the booking appointment. However, the majority of infections during this outbreak were confirmed in pregnancies >20 weeks, and all of the 42 pregnancies monitored had uneventful outcomes. The data reviewed for this outbreak period would suggest the underinvestigation of early gestation infection and the underestimation of early gestation loss.

Eight women were confirmed with early stage B19V infection. One was antibody-negative after contact with a rash but had a B19V DNA load of $1.35 \times 10^{12}$ copies ml$^{-1}$, and seven were IgM-positive at booking, two of whom were IgG-negative. These women posed a potential cross-infection risk to staff and other patients; acute B19V was confirmed in an antenatal clinic midwife and appeared work-related. In an outbreak, women attending clinics may therefore pose an infectious risk and, where under investigation following exposure, should ideally have bloods taken at home by a B19V-immune midwife.

European Directive 92/85/EEC requires individual states within the European Community to afford protection to pregnant women in the workplace against biological agents harmful to the developing fetus (The Council Of The European Communities, 1992). The National Institute for Health and Clinical Excellence (NICE) also advises that occupation should be ascertained to identify those at increased risk through occupational exposure (NCCWCH, 2008). In this review, occupation was rarely available, but in a linked audit was established as a risk factor for over 50 % of the women reviewed, the majority of whom were teachers or day care workers. In B19V epidemics, the risk to susceptible pregnant women is closely related to their level of contact with children (Gilbert, 2000; Harger et al., 1998; Valeur-Jensen et al., 1999). The risk of infection is
considered to be highest for women with an infected child in the home, the risk increasing with the number of children present. Whilst not all well-designed studies demonstrate an occupational risk (de Villemeur et al., 2011; Stelma et al., 2009), others clearly do (Adler et al., 1993; Gillespie et al., 1990), including a large Danish study involving over 30,000 serum samples (Valeur-Jensen et al., 1999), which confirmed that the intensity of exposure to children at work is a significant risk factor for acute B19V infection. The cost in financial terms of antenatal screening for B19V is thought excessive (Gaertner et al., 2007), but that cost analysis may be underestimating the true burden of fetal loss and discounting the potential financial impact of occupational leave for non-immune women. As suggested by this study the lack of reliable investigation and therefore of reliable public health reporting of B19V fetal loss, especially in early pregnancy, makes the discussion poorly supported and leaves those women most affected uninformed.

In the face of regular and predictable outbreaks and attendant pregnancy losses, there is little evidence throughout Europe of any consistent public health attempt to reduce the burden of this disease. In the UK, B19V is not included in the antenatal screening infectious diseases programme, which leaves it mostly unaddressed by those healthcare professionals who deliver it. Improvements in diagnosing B19V should be used to underpin (a) the education of both healthcare providers and women seeking medical attention when exposed to a rash, and (b) general practitioners in appropriate management of those self-referring. It should also be the starting point for a more robust collection of data by public health agencies, which is currently sadly lacking, and which should be informing the debate for vaccine development. As a minimum, during B19V outbreaks and where a booking blood has been stored, the follow-up of miscarriages and stillbirths should include the taking of a maternal blood to confirm or exclude B19V as a cause of fetal loss. In the meantime, addressing ways to lessen exposure should be a priority, including reducing the risks of occupational infection.

ACKNOWLEDGEMENTS

We would like to thank Danny McWilliams, Belfast Trust Information Services.

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