Outbreak investigation and epidemiology - from practice to science -.
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CHAPTER 3

Confirmation of an outbreak of parvovirus B19 in a primary school using IgM ELISA and PCR on thumb prick blood samples

3.1 ABSTRACT

This chapter discusses the confirmation of an outbreak of parvovirus B19 in a primary school using IgM ELISA and PCR on thumb prick blood samples. Although parvovirus infections are usually benign and self-limiting, it is important to confirm the diagnosis in a public health setting, which might involve pregnant women for whom an outbreak could lead to medical consequences. In these situations, microbiological confirmation by thumb prick is a relatively low-invasive method that is simple to carry out.

Because relatively small blood volumes are obtained in thumb prick blood samples, we compared the results of two different techniques during an outbreak of erythema infectiosum: the usual serological detection of IgM antibodies (ELISA) versus PCR-based detection of viral DNA.

In a school-based outbreak, 39 cases (33 schoolchildren, three parents, three pre-schoolers) were registered over a period of 11 weeks. Sera were obtained from 23 of the school cases and two of the three parent cases. Of all thumb prick serum samples, 65% (15/23) tested positive or borderline positive for parvovirus IgM with ELISA, while 70% (16/23) tested positive or borderline positive with PCR. Although the overlap between the two tests was large (11 samples tested positive or borderline positive in both), a substantial number of samples showed contradictory results (9 samples).

The overall picture of 37 clinical cases of erythema infectiosum and two adult cases with arthritis, linked to a primary school, fits in well with positive diagnostic results by either technique for parvovirus B19, convincingly demonstrating an outbreak of fifth disease. The considerable number of discrepancies in sample results demonstrates that maximum sensitivity of parvovirus testing would require both tests to be performed.
3.2 INTRODUCTION

Although parvovirus infections are usually benign and self-limiting, it is important for public health to confirm the diagnosis if pregnant women are involved for whom an outbreak could have medical consequences. Between January and May 2001, the MHS was informed of 14 clusters of exanthematous illness (involving 2 to 33 cases, with a mean of 7 cases). Cases were reported among children in 10 of the 125 local primary schools in the region and in 4 of the 130 day-care centres. The clinical picture was diagnosed as erythema infectiosum (fifth disease) because of the frequently reported ‘slapped cheek’ redness and ‘lace-like’ exanthema, although general practitioners also mentioned scarlet fever and rubella in some cases. We investigated one outbreak linked to a primary school, the examination being prompted by the persistent occurrence of cases and an unusual level of worry among the general public and especially among pregnant women. Laboratory diagnosis of outbreaks like this is usually hampered by unease among parents, who often refuse to allow venepuncture in their children. In these situations, the use of blood obtained by thumb prick for laboratory confirmation is a less invasive procedure and is simple to carry out. Therefore, the purpose of the present study was to test the acceptability of using thumb prick blood sampling in the investigation of a suspected outbreak of parvovirus B19 in a primary school. Furthermore, as relatively small blood volumes are obtained by thumb pricks, it was decided to compare the results of two different analytical techniques: the usual serological detection of IgM antibody versus PCR-based detection of viral DNA.

3.3 METHODS

Epidemiology

The school was visited in April 2001, and class registers were obtained to map out the outbreak. At the time of this visit, five children showed redness of the face (slapped cheek) and lace-like exanthema on the skin, clinically suggesting erythema infectiosum.

Information

On the same day, the consultant for communicable disease control informed the general practitioners in the area about the regional epidemiology of the disease, the specific outbreak, the disease in general and how to counsel pregnant women. Information was provided on the estimated low risk of complications (a 2% risk with approximately 40% of pregnant women susceptible, a 50% risk of transmission in family contacts and a 10% risk of abortion or intra-uterine fetal death in first 20 weeks of pregnancy)\(^1\) and on the possibility for pregnant women to have antibody tests, according to the Dutch guideline for erythema infectiosum prepared...
by the National Co-ordination Centre for Communicable Disease Control (LCI). The schoolchildren and staff were informed by letter about the consequences of an outbreak of erythema infectiousum.

**Inventory**

A list of recently affected pupils and staff was compiled with the help of the teachers and the headmaster. Children with a recent history (in the past few months) of exanthema and adults with exanthema or arthritis were included as cases (case definition). Parents of affected pupils were contacted by telephone for a brief interview including questions about their sex, age, nature of symptoms and exanthema, first day of onset of the symptoms and exanthema, duration, known other (family) cases, rubella vaccination status, general practitioner and possible transmission to contacts.

**Thumb prick blood sampling and diagnostic techniques**

Each parent was asked to give informed consent for the taking of blood samples from the affected children by thumb prick. An intravenous blood sample was taken from adult patients. Serum samples were tested for IgM antibodies to parvovirus using the Biotrin Parvovirus B19 IgM enzyme immunoassay (Biotrin, Dublin, Ireland), essentially as described by the manufacturer. The only modification involved the specimen preparation, in which only 0.5 ml of diluent was used, with 5 μl of serum, instead of the prescribed 1 ml sample diluent and 10 μl serum. This means that concentrations remained unaffected, as only 100 μl of the preparation is used for each assay. After serology, the remainder of the serum samples were subsequently tested for the presence of parvovirus DNA, after DNA extraction by means of the MagnaPure system (Roche Molecular Systems). Two adult venepuncture samples were not tested with PCR as the interest of this study was in thumb prick blood samples. The extraction volume was always set at 200 μl. In cases where the serum sample volume was smaller, this was adjusted by the addition of saline, while recording the original volumes. The PCR method has been modified from the procedure originally described by Salimans et al. and has been used in the routine diagnostic laboratory for many years. The primers in this single round PCR (biotinylated V021s ACTGGTGCGCTTACTACG and V022as TAACCCCTCTACACACTG) have been derived from the NS1 gene and generated an amplified product of 248 bp (nt 497-744 according to Shade et al.). The results were confirmed by hybridisation in an EIA format with a DIG labelled probe V023 GCAGTGGCTCTAAGCTT. This PCR detects less than five copies of parvovirus B19 DNA, which has been determined using a quantitative parvovirus B19 assay (Lightcycler, Roche). Therefore the sensitivity of the assay is less than 250 copies/ml blood.
3.4 RESULTS

Epidemiology

In this outbreak, 39 cases (33 schoolchildren, three parents, three pre-school children) were registered over a period of 11 weeks (no new cases presented after this period). None of the staff members were pregnant or had symptoms (0/12). Of the 230 schoolchildren, 33 were registered as affected by the illness (i.e., an attack rate (AR) of 14%). Three parents had symptoms, of whom two had arthritis and one had erythema only. We registered only eight susceptible pre-schoolers in the affected families, of whom three were also involved in the outbreak (AR 38%: 3/8). The mean age of the affected schoolchildren was 7.1 years (s.d. 1.9 years). This was slightly younger than the mean age of the children in the school as a whole: 7.8 years (s.d. 2.5 years). Similarly, the AR was slightly higher in the lower age groups, but children in all grades were affected. As shown in figure 3.1, the first case presented during the fifth week of 2001. There were no new cases after the pupils returned from their Easter holiday (week 16). All affected children had been vaccinated with the vaccine for mumps, measles and rubella at 14 months, which made rubella unlikely as a cause of this outbreak.

Thumb prick blood sampling

One affected parent, the three affected pre-schoolers and four newly reported cases in week 15 were not eligible for blood sampling, as blood sampling took place in the beginning of week 15 on the primary school. Sera were obtained of 23 of the 29 school cases (79%) and 2 of the 3 parent cases in week 15. The thumb prick was well tolerated (two children cried) and well accepted. Parents of two pupils could not be contacted for informed consent and one parent with three affected children refused.

Diagnostic techniques

Of all blood samples, 64% (16/25) tested positive for parvovirus IgM with ELISA, while one sample yielded a borderline value. The PCR test yielded 61% (14/23) positive tests for parvovirus B19, with two samples yielding a borderline value. Although the overlap between the two tests was large (10 samples tested positive in both), a substantial number of samples showed contradictory results, as is shown in table 3.1. If the borderline values are regarded as positive, 5 samples tested positive only by PCR and 3 samples tested positive only by ELISA. Three samples tested negative for both.
FIGURE 3.1. Epidemic histogram of affected cases by onset of exanthema or arthritis.

Table 3.1. Results of detection of IgM-antibodies (ELISA) and viral DNA (PCR) in serum samples (percentage out of a total of 23 thumb prick serum samples).

<table>
<thead>
<tr>
<th>Test</th>
<th>ELISA positive</th>
<th>ELISA negative</th>
<th>ELISA borderline</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not tested PCR*</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>PCR positive</td>
<td>10 (43%)</td>
<td>4 (17%)</td>
<td>-</td>
<td>14</td>
</tr>
<tr>
<td>PCR negative</td>
<td>3 (13%)</td>
<td>3 (13%)</td>
<td>1 (4%)</td>
<td>7</td>
</tr>
<tr>
<td>PCR borderline</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>8</td>
<td>1</td>
<td>25</td>
</tr>
</tbody>
</table>

* Two venepuncture serum samples of adults (no thumb prick blood samples)

3.5 DISCUSSION

The overall picture of 37 clinical cases of erythema infectiosum among children and two adult cases with arthritis, all linked to a primary school, fits in well with the positive diagnostic results for parvovirus B19 obtained by both techniques in 88% (22/25) of the samples tested, convincingly demonstrating an outbreak of fifth disease. The epidemic histogram shows the expected pattern of person-to-person transmission with a number of peaks separated by the time from acquisition until rash illness occurs (two to three weeks; the incubation period is one week: from time of acquisition until viraemic and the co-incidental flu-like illness). After the highest peak in week 14, the Easter holiday interrupted the outbreak in week 16.
The outbreak pattern is similar to those previously reported for other school outbreaks of fifth disease. A 3 to 4 yearly cycle of parvovirus B19 is apparent in the Netherlands, although the differences in case numbers are relatively small. According to the Sentinel System of the Dutch Working Group for Clinical Virology, 1998 was an outbreak year, 1999 and 2000 had a lower number of cases and 2001 showed an increase again. Probably, 2001 and the outbreak as studied represented the beginning of a period with increased parvovirus B19 activity which fits well in the reported periodicity. The study demonstrates that the thumb prick was well tolerated and well accepted as a low-invasive method to collect blood samples in primary school children during outbreaks. Salivary IgM and IgG assays as public health diagnostics for parvovirus B19 are not available in the Netherlands, though this completely non-invasive method might even be more widely acceptable. Unfortunately, however, the sensitivity of this method compared to blood testing has proved disappointingly low (60%).

The present comparison of two completely different diagnostic techniques, PCR and ELISA, provided some interesting results. Both techniques were able to identify parvovirus B19 as the etiological agent in this outbreak, although neither technique was clearly superior in terms of sensitivity, both testing positive in about equal numbers of subjects (table 3.1). There was a considerable number of discrepancies, in which only one test was positive (9 cases versus 11 yielding the same positive or borderline positive results), demonstrating that maximum sensitivity for parvovirus testing would require both tests to be performed. However, the samples received for PCR analysis were far from optimal. Three discrepant PCR negative, ELISA positive results may be attributed to sample limitations. In those samples only minute amounts of serum were available for DNA extraction and it is likely that in an optimised procedure with 200 μl of serum, the PCR would be more sensitive. Another possibility is that the PCR negative samples are true negatives. This would imply a rather large individual variability with regard to the persistence of IgM antibodies and viral DNA. It is not evident from our results that differences between the results of the two techniques can be explained by time intervals after the onset of the disease: DNA was found in one of the earliest cases (with onset of symptoms ten weeks prior to sampling) and was absent from two late cases in which IgM was already present (with onset of symptoms two weeks prior to sampling). Practical feasibility largely determined the design of the study and the methodology used, in terms of the size and nature of the blood samples. Despite these shortcomings, both assays are capable of labeling an outbreak as parvovirus B19 related.

It may be concluded that a diagnosis based on PCR is an additional tool for public health services to study parvovirus outbreaks, as it can identify additional cases if used together with the usual serological tests. The PCR technique alone is also able to provide sufficient evidence for the cause of the outbreak, being fully comparable to the serological approach. A comparative study with an optimal design for both assays would be necessary to determine whether combined application is necessary if maximum sensitivity is required in certain
clinical situations or when recent parvovirus infection has to be excluded for organ donation purposes.

3.6 REFERENCES

Drie keer meningitis in één klas

GGD vermoedt verband met verbluffend handbaltoernooi

Nekkramp in Landgraaf

Eenige zekerheid

Drie leerlingen van de St. Lambertusschool in Limburg overstelpen met honderden telefoontjes van bezorgde ouders. Dat hier een bijzondere situatie is, staat vast, ook al liet de GGD Zuid-Limburg een echter.