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Oral fluid: Non-invasive alternative for parvovirus B19 diagnosis?

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ABSTRACT

Background: Infections with parvovirus B19 (B19V) have been associated with a wide range of disease manifestations of which erythema infectiosum (fifth disease) in children is most common. Clinical signs following infection of children with B19V can be similar to measles and rubella. Laboratory detection of B19V infections is based on detection of B19V-specific IgM antibodies by enzyme immunoassay (IgM-EIA) and/or B19V DNA by quantitative PCR (qPCR) on blood samples. The need for invasive sampling can be a barrier for public health diagnostics.

Objectives: To evaluate the use of a dual target B19V-qPCR directed against the NS1 and VP2 of B19V on oral fluid samples as a non-invasive alternative for laboratory diagnosis of B19V infections in children below 12 years of age with exanthema.

Study design: Oral fluid and serum samples were collected from 116 children with exanthema. All serum samples were tested by IgM-EIA/IgG-EIA, while all oral fluid and 56 serum samples were tested by B19V-qPCR.

Results: B19V-specific IgM antibodies were detected in 25 of 116 children in the study. B19V DNA was detected in oral fluid in 17 of the 25 children who were IgM positive, as well as two children who were IgM-equivocal or negative. The child with the equivocal IgM had a high quantity of B19V DNA in oral fluid (7 log IU/ml), compatible with an acute B19V infection. The IgM-negative child was IgG-positive and 4 log IU/ml B19V DNA was detected in the oral fluid sample, suggesting an acute infection and a falsely negative IgM. Sample size calculations indicated that oral fluid samples for qPCR should be collected from 2 to 3 children during outbreaks of exanthema to achieve similar sensitivity as IgM-EIA for one child (≥ 0.9) to confirm or exclude B19V.

Conclusions: Results indicate that oral fluid samples are a suitable public health alternative for detection of B19V infections, potentially lowering the barriers for sampling.

1. Background

Infection with parvovirus B19 (B19V) causes erythema infectiosum in children (so called “slapped cheek disease” or” fifth disease”) and arthropathies in adults, although 25–50% of infections are subclinical [1–3]. In pregnant women, transplacental transmission of the virus to the fetus can result in hydrops fetalis, fetal or congenital anemia, abortion, or stillbirth [1,4,5]. Furthermore, various complications have been described following infection with B19 V [1].

Diagnosis of B19V infections in children with exanthema is of interest since B19V infections in children pose a risk for transmission to pregnant mothers [5]. In addition, B19V is one of the viruses that can cause exanthema in children, similar to rubella virus and measles virus, and inclusion of B19V detection in a viral panel for diagnosis of exanthemas would result in an increase in the proportion of cases with a positive diagnosis [6–8].

Laboratory diagnosis of acute infections with B19V is currently based on serological assays, such as enzyme immunoassays (EIA) to detect B19V-specific IgM and IgG antibodies, and molecular assays, to detect circulating B19V DNA in blood samples. Various studies have demonstrated that a combination of these two provided the most sensitive diagnosis of B19V infections [9–13]. In most cases, a positive IgM response in combination with clinical signs of B19V infection will provide an accurate diagnosis of a case of fifth disease. In some cases,

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collection of the blood sample might have been too early to detect a positive B19V-IgM response, while B19V DNA can already be detected in a blood sample. However, B19V DNA can remain present in the blood stream for at least several months after infection. Therefore, a clear link between the onset of clinical disease (exanthema) is important for the interpretation of the laboratory results [14,15].

However, often no samples of suspected cases are collected from children with exanthema. The availability of a non-invasive sampling method might increase the willingness of children and their parents to provide a sample for diagnostics. Collection of a fingerprick blood sample can be considered a first alternative to collect serum samples for diagnostics [9]. However, this is still perceived as slightly invasive and requires medical assistance.

Oral fluid sampling might be a suitable, non-invasive, easy-to-use alternative for diagnosis of acute B19V infections. Quantitative reverse transcriptase PCR (RT-qPCR) is already routinely performed on oral fluid for measles and rubella virus and detection of B19V DNA by qPCR on oral fluid samples could be an integral part of laboratory diagnostics currently performed for diagnosis of outbreaks of exanthema [16].

2. Objectives

The objective of this study was to evaluate the use of qPCR on oral fluid samples for the diagnosis of B19V infections in children with non-vesicular exanthema in a direct comparison with IgM antibody detection in serum. The obtained sensitivity (and specificity) of the B19V-qPCR was used to calculate the number of children that needs to be sampled for detection of B19V as the causative agent of an outbreak of exanthema for public health purposes.

3. Study design

3.1. Selection of clinical cases

Samples were collected from children attending day care and primary school (maximum 12 years of age) within maximum 14 days after development of a maculopapular rash (exanthema). Collection of samples was performed within the context of a Dutch surveillance protocol for laboratory diagnosis of infections with measles, B19V or rubellavirus in children with exanthema. To prevent missing children with especially rubellavirus infection, the inclusion criterion for submitting samples for laboratory diagnostics was limited to the presence of an exanthema only. Samples were used that were collected from children during two periods, between December 2003 and July 2005 (group 1) and between March 2010 and January 2018 (group 2). Large outbreaks of rubella and measles virus occurred in the Netherlands from September 2004 until July 2005 [17] and from May 2013 until March 2014 respectively [18]. Informed consent to obtain clinical specimens for laboratory diagnosis and additional test validations was obtained from the parents.

3.2. Sample and data collection

From children with exanthema, an oral fluid and a blood sample were collected. Oral fluid samples were collected using an oral fluid collection system containing a sponge (Oracol, Malvern Medical Developments). Blood samples from the first group were collected on dried blood spot cards (Protein Saver™ 903™, Whatman), while blood samples from the second group were collected in Microtainer tubes (Becton Dickinson).

Samples were submitted to the diagnostic laboratory of the Netherlands National Institute for Public Health and the Environment (RIVM). Upon arrival, samples were stored at + 4 ºC until further processing for laboratory diagnosis of measles and rubella virus infection by RT-qPCR and B19V infection by IgM-EIA and IgG-EIA. Remaining sample material was stored at -70ºC until use for detection of B19V DNA by qPCR.

From each child, the date of onset of prodromal symptoms and/or the date of onset of exanthema, the date of sample collection and date of birth were registered.

3.3. Molecular diagnostics

Nucleic acids were extracted from oral fluid and serum samples using the MagNA Pure96 (Roche) according to the protocol of the manufacturer. Oral fluid samples were subsequently tested for the presence of measles virus RNA and rubellavirus RNA by RT-qPCR essentially as described previously using TaqMan Fast virus One-step Master Mix on a Roche LC480 platform [16].

In addition, oral fluid samples and serum samples stored in microtainer tubes (group 2) were tested for the presence of B19V DNA using a multiplex dual-target qPCR with primers and probes targeting the partial NS1 and VP2 genes as described previously [19]. B19V qPCR on serum and oral fluid were performed using TaqMan Fast virus One-step Master Mix on a Roche LC480 platform. Oral fluid and serum samples were considered positive when a clear positive signal was detected with either or both the NS1 and VP2 qPCR. Samples with a discordant result between the two qPCRs and a relatively high qPCR Ct-value, were re-tested.

3.4. Serological diagnostics

Fingerprick blood samples from group 1 were collected on dried blood spot cards and were harvested according to a standard protocol. Fingerprick blood samples from group 2 were collected in Microtainer tubes and tubes containing clotted blood were centrifuged and serum was harvested. The obtained serum was subsequently tested for the presence of B19V-specific IgG and IgM antibodies using a B19V-specific IgG and IgM EIA (Biotrin International) according to the instructions of the manufacturer.

3.5. Statistical analysis

The correlation between the obtained qPCR targeting the partial NS1 and VP2 (IU/ml) genes and the correlation between the obtained qPCR results and the interval between collection date and date of onset of exanthema were calculated using Pearson correlation coefficient (r). Statistical analysis of proportions of $qPCR^{pos}IgM\text{-}EIA^{pos}$/ $IgM\text{-}EIA^{pos}$ samples of both groups was performed using Fisher's exact test in R version 3.4.3 [20]. Sensitivity and specificity of the qPCR were calculated using IgM-EIA as the reference standard.

3.6. Evaluation of sample size collection

The calculated sensitivity of the qPCR was used to evaluate how the qPCR could be implemented for diagnostics to detect B19V as the causative agent during an outbreak of exanthema among children in a public health setting. If B19V can be excluded in case an outbreak is caused by another pathogen is indicated by the specificity of the qPCR. The chance of detection of a B19V positive sample (P_{detect} $X > 0$) during an outbreak of B19V (e.g. at a day care centre) was calculated for both the IgM-EIA and qPCR assuming that samples collected during an outbreak of exanthema at the same time and at the same location will involve only a single pathogen. Thus, the prevalence of B19V during an outbreak of this virus was estimated to be 90–100% in the collected samples if samples are collected from children with exanthema only. A somewhat lower prevalence of B19V in the samples might be expected if clinical signs are not very clear in all children from whom samples were collected. Therefore, P_{detect} $X > 0$ was calculated for sample prevalences of 80, 90 and 100% and a sample size ranging from one to four samples using the following formula in R version 3.4.3 [20]:

P_{detect} = 1-(1-prevalence)^n (for IgM-EIA)

P_{detect} = 1-(1-sensitivity*prevalence)^n (for qPCR)

4. Results

4.1. Study population

In total 116 cases of children with exanthema were included in this study. The first group consisted of 60 children (mean age 5.2 years) and the second group of 56 children (mean age 4.1 years).

4.2. Detection of measles and rubella virus by RT-qPCR

Infection with rubellavirus was confirmed in 21 children by RT-qPCR of the first group, while no measles or rubellavirus was detected by RT-qPCR in samples collected from the other children of both groups.

4.3. Detection of B19V infection(s) by serology

B19V-specific IgM antibodies were detected in 25 of 116 investigated children, while two samples were considered equivocal (Table 1). The youngest child with a positive B19V-specific IgM response was 2.8 years of age. B19V-specific IgG antibodies were detected in 49 of 114 tested children and results from 7 samples were considered equivocal (Table 1). From two children, serum could not be tested for the presence of B19V-specific IgG antibodies since the amount of available serum from these children was very limited. In all samples that tested positive for the presence of B19V-specific IgM antibodies, also B19V-specific IgG antibodies were detected (Table 1).

4.4. Detection of B19V infection(s) by qPCR

B19V was detected in 19 of 116 oral fluid samples by qPCR comprising the children of both group 1 and group 2 (Table 2). Within group 2, B19V DNA was detected in 18 of 56 tested serum samples. For 11 of these children (61%), B19V DNA was also detected in oral fluid (Tables 2, 3 and S1, Fig. 1).

In 17 out of 18 qPCR positive serum samples, B19V DNA was detected by both qPCRs directed against NS1 and VP2, while in one case B19V DNA was detected in serum by qPCR directed against VP2 only. In total 16 out of 19 positive oral fluid samples tested positive for the presence of B19V DNA by both qPCRs directed against NS1 and VP2, while in three cases B19V DNA was detected by qPCR targeting either NS1 or VP2 (data not shown). The Pearson correlation coefficient *r* between the two qPCRs for oral fluid samples was 0.93 (Fig. S1).

The mean quantity of B19V DNA detected in positive oral fluid samples was 3.9 ^{10}log IU/ml (S.D. 1.5 ^{10}log IU/ml) and 3.9 ^{10}log IU/ml (S.D. 1.4 ^{10}log IU/ml) for the NS1-qPCR and VP2-qPCR respectively. The mean quantity of B19V DNA detected in positive serum samples was 2.8 ^{10}log IU/ml (S.D. 0.8 ^{10}log IU/ml) and 3.5 ^{10}log IU/ml (S.D. 1.2 ^{10}log IU/ml) respectively. There was no linear relationship between the quantity of B19V DNA and the number of days between collection and onset of exanthema, in both serum and oral fluid samples (*r*-values ranging from 0.005-0.011) (Fig. S2).

Table 1
Combination of laboratory results of IgM-EIA and IgG-EIA.

Test	Result	IgM-EIA			
		Positive	Equivocal	Negative	*Total*
IgG-EIA Group 1	Positive	8	0	20	28
	Equivocal	0	0	3	3
	Negative	0	1	27	28
	Total	*8*	*1*	*50*	*59*
IgG-EIA Group 2	Positive	17	0	5	22
	Equivocal	0	0	4	4
	Negative	0	1	28	29
	Total	*17*	*1*	*37*	*55*

Table 2
Overview of all laboratory results for all children in this study subdivided by their laboratory results.

Group	IgM	IgG	qPCR serum	qPCR oral fluid	Number of children
1	+	+	n.d.	+	6
	+	+	n.d.	–	2
	equiv.	–	n.d.	+	1
	–	+	n.d.	+	1
	–	+	n.d.	–	19
	–	equiv.	n.d.	–	3
	–	n.d.	n.d.	–	1
	–	–	n.d.	–	27
Total					*60*
2	+	+	+	+	11
	+	+	+	–	6
	equiv.	–	–	–	1
	–	+	–	–	4
	–	–	+	–	1
	–	equiv.	–	–	4
	–	n.d.	–	–	1
	–	–	–	–	28
Total					*56*

n.d.: no data.
equiv.: equivocal EIA results.

Table 3
Combination of results of IgM-EIA and qPCR of both groups.

qPCR sample	Laboratory results		Number of children
	IgM	qPCR result	
Oral fluid	Positive	Positive	17
	Positive	Negative	8
	Equivocal	Positive	1
	Equivocal	Negative	1
	Negative	Positive	1
	Negative	Negative	87
	Total		*116*
Serum	Positive	Positive	17
	Positive	Negative	0
	Equivocal	Positive	0
	Equivocal	Negative	1
	Negative	Positive	1
	Negative	Negative	37
	Total		56

4.5. Comparison of detection of B19V infections by IgM-EIA and qPCR

In six of the eight children (75%) with B19V-specific IgM antibodies also B19V DNA was detected by qPCR in oral fluid in the first group and in 11 of the 17 children of the second group (65%). Since results of both groups were similar (Fisher's exact test p = 0.67), results of both groups were combined (Table 3; sensitivity 68%). The qPCR on oral fluid was negative for 89 out of 91 IgM-EIA negative/equivocal children (specificity 98%). No children that tested positive for rubellavirus infection by RT-qPCR tested positive for B19V infection by IgM-EIA and qPCR.

B19V DNA was detected in 18 serum samples, 17 of these serum samples tested also positive by IgM-EIA (sensitivity 100% of qPCR on serum) (Table 3, Fig. 2). One child had relatively low Ct-values (mean Ct-value 0.85 ^{10}log IU/ml) without a positive IgM-EIA and IgG-EIA test result.

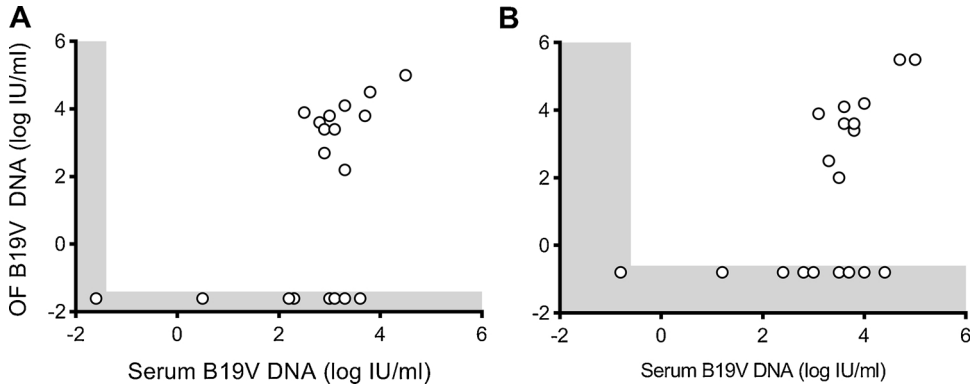

Fig. 1. Associations between quantitative B19V-qPCR results in serum and oral fluid. Oral fluid (OF) samples from all children and serum samples from children of group 2 were tested both by NS1 (A) and VP2 (B) B19V-qPCR. Collected qPCR results from individual cases are indicated in IU/ml.

4.6. Estimation of sample size collection during an outbreak of B19V

P_{detect} correlated with the prevalence and was higher for IgM-EIA than for qPCR. With a prevalence of 0.8 at least 3 children needs to be sampled for qPCR and at least 2 children needs to be sampled with IgM-EIA to detect a B19V infection with ≥90%, while with a prevalence of 1 only 2 and 1 children should be sampled respectively (Fig. 3).

5. Discussion

In the present study, the use of oral fluid samples for molecular detection of B19V in children with exanthema was evaluated as a non-invasive alternative for fingerprick blood sampling. Comparison of results from B19V-qPCR on oral fluid with B19V-IgM-EIA on serum revealed that detection of B19V DNA in oral fluid is possible although with lower sensitivity (68%) compared to the IgM-EIA. Only children were included from which samples were collected within 14 days of onset of exanthema. Therefore we anticipated that a case with exanthema and a positive B19V-IgM-EIA result was a child with a recent B19V infection (fifth disease). In addition, all IgM positive cases of the second group could be confirmed by detection of B19V DNA in serum in our study.

Both sensitivity and specificity are likely somewhat underestimated as two children with negative/equivocal B19V-IgM-EIA results tested clearly positive in the qPCR on oral fluid. Samples from one child were collected one day after the onset of exanthema. A high copy number of B19V DNA was detected in oral fluid (7.0 and 7.5 log IU/ml in the NS1 and VP2 qPCR respectively), while B19V-IgM-EIA results were equivocal. It was presumed that samples from this child were collected too early for adequate antibody detection. Samples from the other child were collected four days after the onset of exanthema and B19V-IgG antibodies were detected in serum. Since B19V DNA was clearly detected in oral fluid by both B19V-qPCRs (4.5 and 4.3 log IU/ml in the NS1 and VP2 qPCR respectively), it was presumed that the results of the B19V-IgM-EIA were false negative and that this was a child with an ongoing infection or possibly reactivation. Therefore, both children

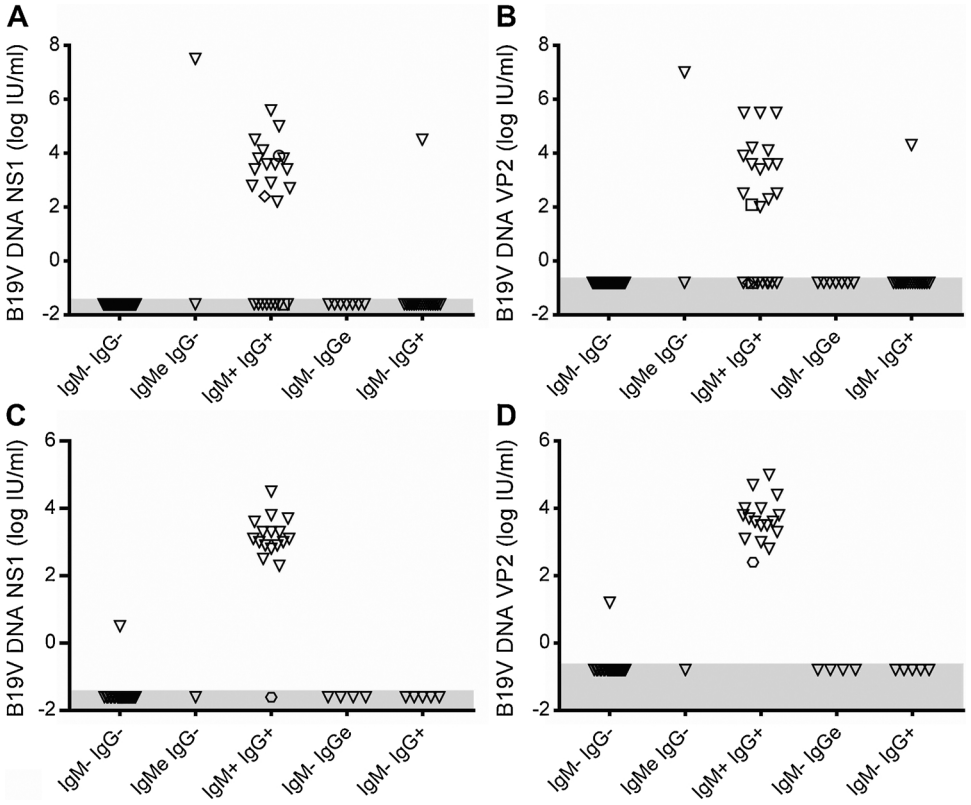

Fig. 2. Associations between quantitative B19V-qPCR and B19V-IgM/IgG-EIA results. Oral fluidsamples from all children (A, B) and serum from samples of group 2 (C, D) were tested for the presence of B19V DNA by a NS1 (A, C) and VP2 specific qPCR (B, D). Data from obtained qPCR results were presented with data from individual B19V-IgM/IgG-EIA (negative [−], positive [+], or equivocal [e]). The light grey areas indicate the values where qPCR results were considered negative. Data from all individual children are indicated by triangles. Laboratory results of children with a discrepancy between the NS1 and VP2 qPCR are indicated with different symbols.

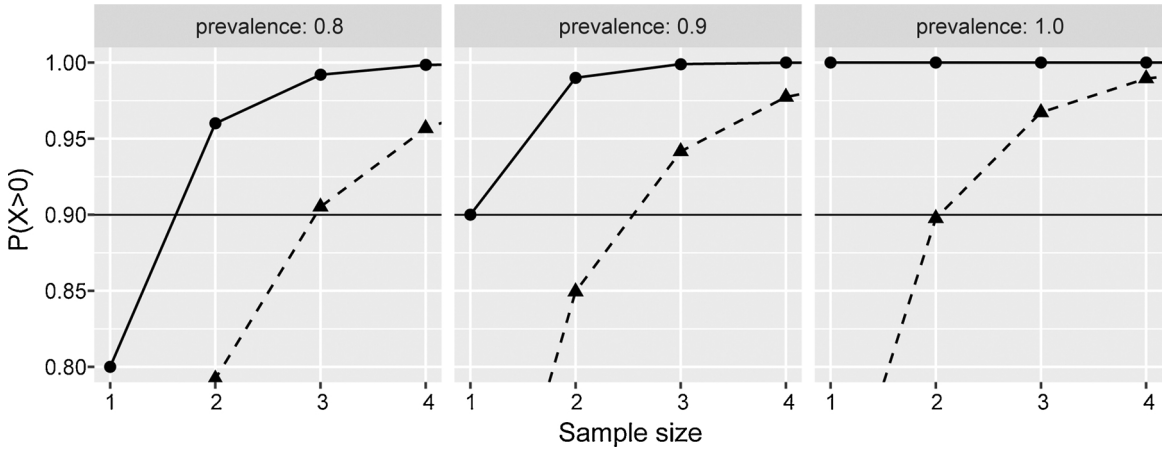

Fig. 3. Relationship between sample size, prevalence of B19V in samples and the chance of detection of B19V infection during an outbreak of B19V using both B19V-IgM-EIA (solid line) and B19V-qPCR dashed line).

were considered as cases of fifth disease. On the other hand, in serum from one child a relatively low amount of B19V DNA was detected in the absence of B19V-specific IgM and IgG antibodies and it was presumed that this was a false positive B19V-qPCR result [21].

The results of our study confirm the results of previous studies, which demonstrated that the most sensitive diagnosis of fifth disease is based on both qPCR and IgM-EIA [9–13,21]. When the two cases with a equivocal/negative IgM and clear positive qPCR result are indeed included as B19V cases to calculate a composite reference standard based on the combined results of qPCR on oral fluid and IgM-EIA, the sensitivity of the qPCR on oral fluid would increase to 70% [22].

Of interest, oral fluid has been used previously for detection of B19V-specific IgM and IgG, but the sensitivity was lower compared to serum and also slightly lower (60%) than the qPCR on oral fluid as used in this study [23]. In addition, oral fluid was chosen instead of a nasal or pharyngeal swab for detection of B19V DNA since it was easiest to obtain and already common practice for detection of measles and rubellavirus by RT-qPCR.

The relative low sensitivity of the qPCR on oral fluid compared to the IgM-EIA could be explained by the limited or short period of excretion of B19V via the respiratory tract, as shown previously for B19V infected adults [24,25]. Therefore, we limited the analysis to samples that were collected within 14 days of onset of exanthema.

The clinical criterion for collection of samples was the presence of exanthema only. Although infections with rubella virus and B19V could explain the exanthema in a proportion of the children, the cause of exanthema in the majority of the children remained unclear and could be of non-infectious etiology. However, an infectious cause was suspected in case more children presented exanthema at the same time [26,27].

Among the children that tested positive by B19V-qPCR in oral fluid, three different epidemiological clusters could be defined based on available metadata (date of sample collection, place, and school). Two clusters consisted of two children each. In these clusters, results of B19V-IgM-EIA and B19V-qPCR on serum were positive in both children, while B19V DNA could be detected in one oral fluid sample of each cluster. The third cluster consisted of four children. Results of B19V-IgM-EIA and B19V-qPCR on serum were also positive in all four children, while B19V DNA could be detected in oral fluid in three children. In one of these children B19V DNA could be detected by qPCR against only one target (NS1).

In conclusion, results of the present study indicate that molecular detection of B19V on oral fluid is a suitable non-invasive alternative for public health diagnostics to detect or exclude B19V as the causative agent of outbreaks of exanthema among children at a day-care-centre or school.

Author contributions

Design of the study: Rob van Binnendijk; performed experiments: Jeroen Kerkhof, Jeroen Cremer, Daphne Gijselaar; analyzed data: all authors, preparation of first draft version of the manuscript: Rogier Bodewes, Rob van Binnendijk, contribution to final draft: all authors.

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Competing interests

None declared.

Ethical approval

Not required.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2019.05.008.

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