Detection of human parvovirus B19 in serum samples from children under 5 years of age with rash–fever illnesses in the Democratic Republic of the Congo

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ARTICLE INFO

ABSTRACT

Background: It has been demonstrated that infection with human parvovirus B19 (B19V) is associated with rash–fever illnesses. The present study aimed to investigate B19V as an aetiological agent of rash–fever syndromes in Congolese children confirmed as measles and rubella IgM-negative. An ELISA IgM test and PCR were performed to screen for B19V.
Methods: A total of 177 archived serum samples were randomly selected from the measles biobank of the National Institute for Biomedical Research (INRB). Samples were investigated for anti-B19V IgM and B19V DNA. These samples originated from children <5 years of age with measles-like rashes, previously confirmed as negative for both measles and rubella IgM.
Results: Out of 177 serum samples tested by ELISA and 168 tested by PCR, 109 were positive for B19V IgM antibodies (61.6%) and 87 (51.8%) were positive for B19V DNA. Positive samples in both assays were from all provinces of DRC.
Conclusions: B19V plays a role in rash–fever illnesses in children under 5 years of age suspected of having measles or rubella infections in DRC. As an aetiological cause of rash and fever syndromes, the present study demonstrates that B19V should also be considered during the laboratory investigation of rash–fever illnesses in DRC, particularly in the paediatric population. There is a need to conduct further studies in order to gain a better understanding of the spatiotemporal pattern of B19V and to define the genotype(s) of B19V circulating in DRC.

Introduction

Human parvovirus B19 (B19V) is a small non-enveloped single-stranded DNA (ssDNA) virus that belongs to the *Erythroparvovirus* genus within the *Parvoviridae* family. The virus is widespread in humans, with a prevalence ranging from 10% to 35% in healthy children under 5 years of age (Heegaard and Brown, 2002; Heegaard et al., 2002).

The virus has been associated with different clinical manifestations, such as transient aplastic crisis, chronic anaemia, myocarditis, arthropathy, hepatitis, hydrops fetalis, and rash–fever illnesses (Young and Brown, 2004).

The viral genome consists of 5596 nucleotides (nt) and encodes three proteins, including non-structural protein 1 (NS1) and the two capsid proteins (VP1 and VP2). Phylogenetic analysis of a 994-nt fragment of the NS1/VP1 unique region junction (NS1/VP1u) led to the identification of three genotypes of B19V (1, 2, and 3), with

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no clear difference in clinical outcome between them (Heegaard and Brown, 2002; Young and Brown, 2004).

Despite the implementation of a measles surveillance system and the use of supplementary immunization activities against measles in DRC, there are still notifications of cases with measles-like rashes in recently vaccinated people (Lambert et al., 2000). Skin rashes caused by B19V can be mistakenly reported as measles or rubella infection (Scobie et al., 2015; Oliveira et al., 2001).

The recrudescence of rash–fever outbreaks remains a public health challenge in DRC. Laboratory investigations are limited to measles and rubella IgM, while it is known that some rashes might be caused by other pathogens – mostly viruses such as parvovirus B19, enterovirus, adenovirus, dengue virus, Epstein–Barr virus, chikungunya virus, and human herpes and simplex viruses (Oliveira et al., 2001; Moraes et al., 2011).

The overall incidence of B19V infection is difficult to estimate because most provinces of the country lack vital hospitals, laboratory equipment, statistics, and regional registries.

As recommended by the World Health Organization Regional Office for Africa (WHO/AFRO), the national reference laboratory for measles is focused on measles and rubella IgM tests (WHO, 2017). Parvovirus B19 infections are not notifiable in DRC.

In the Congolese context, with endemic malaria and high prevalence rates of HIV and sickle cell anaemia, B19V can contribute to the severity of these diseases (Toan et al., 2013). Therefore, it is of the utmost importance to define the main characteristics of rash–fever illnesses in all children in DRC.

This preliminary study was conducted to investigate the presence of B19V in serum samples from Congolese children under 5 years of age confirmed as negative for measles and rubella IgM. The findings of this study will provide useful information for settings in which there is recrudescence of rash–fever illnesses despite high vaccination coverage for measles and rubella in DRC. No data on B19V in DRC have been published thus far.

Methods

This study was conducted on archived serum samples collected within 7 days of rash onset from Congolese children under 5 years of age. Serum samples were collected at different sentinel sites of each province of DRC through the measles surveillance system.

All sera originated from hospital outpatients and inpatients with rash and fever that occurred between January and December 2011 in DRC. The samples were shipped to the national reference laboratory for measles located at the National Institute for Biomedical Research (INRB) in Kinshasa for measles and rubella IgM testing. The sera were aliquoted and kept at −20 °C.

According to the database of the national reference laboratory for measles, 2298 serum samples were collected during the study period (January to December 2011). Of these samples, 928 (40.4%) were negative for both measles and rubella IgM. Of these 928 samples, 544 (58.6%) originated from children under 5 years of age. Among the 544 sera confirmed as negative for measles and rubella IgM, 177 were randomly selected and tested for B19V, as shown in Figure 1.

Antibody and viral DNA detection of B19V

The serum samples were screened for anti-B19V IgM antibodies using a commercial ELISA kit (Ridascreen Parvovirus B19 IgM, R-Biopharm, Hesse, Germany). The test was performed according to the manufacturer's instructions.

Viral DNA was extracted from serum samples using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. The extracted DNA was used for the detection of B19V by PCR. Negative controls consisting of phosphate buffered saline (PBS) were extracted concomitantly with sera during the extraction step.

The concentration and purity ratios 260/280 of DNA were immediately determined using a NanoDrop 2000 spectrophotometer. Nine serum samples were not included in the B19V DNA detection because of poor DNA quality (ratio $260/280 > 2$). DNA was amplified based on the protocol published by Koch and Adler, with modifications to optimize the PCR reaction (Koch and Adler, 1990).

Specific primers for a conserved region of the NS1 gene were designed based on the complete genome of B19V (GenBank accession number **NC000883**) (Anon, 2017): forward and reverse nucleotide sequences were NS1-F 5′-TGTAAGATGCAGCCCTGACA-3′ and NS1-R 5′-TTAGAAGCTCCCACATGGCA-3′.

The PCR was performed in a 25-μl reaction mixture containing 5 μl of DNA, 5 μl of 5 × reaction buffer (75 mM Tris HCl (pH 9.0), 2 mM MgCl$_2$, 50 mM KCl, 20 mM (NH$_4$)$_2$SO$_4$, 0.2 mM of dNTPs, and 10 μM each of NS1-F and NS1-R primers. Finally, 0.5 U of Taq DNA polymerase was added. Additional negative and in-house positive controls were included in each PCR run to ensure the absence of carryover contamination and efficiency of the PCR.

Amplification was performed using a GeneAmp PCR system 9700 thermal cycler with the following conditions: an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for
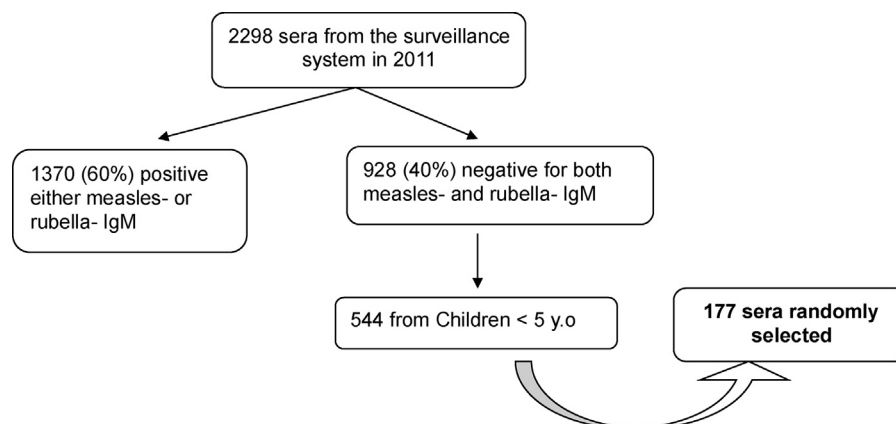
Figure 1. Selection of serum samples. Based on a prevalence of parvovirus B19 IgG antibodies ranging from 10% to 35% in healthy children under 5 years of age, a total of 177 (33%) samples were randomly selected from archived sera at the national reference laboratory for measles.

30 s, 55 °C for 30 s, and 72 °C for 45 s; and a final elongation step at 72 °C for 7 min.

Visualization of the PCR products

The PCR products were separated by electrophoresis on a 1.5% agarose gel (UltraPure Agarose; Invitrogen Life Technologies) in $1 \times$ Tris–borate–ethylenediaminetetraacetic acid (TBE) stained with GelRed nucleic acid stain (Phenix Research Products, Candler, NC, USA). Each well was loaded with 9 μl of amplicons and 1.5 μl of $6 \times$ blue–orange DNA loading dye (Promega, Madison, WI, USA). The gel was run at 200 V for 30 min and the PCR products were visualized under UV light using a gel imager camera (Red Personal Gel Imaging System; Alpha Innotech, USA). The size of positive PCR products was estimated to be around 600 bp by comparing the amplicons with a DNA ladder, as shown in Figure 2.

Data management

The data analysis was performed using SPSS for Windows statistical software version 17.0 (SPSS Inc., Chicago, IL, USA). The Chi-square test was performed to test the significance of variables. A p-value of <0.05 was considered statistically significant.

Results

Out of 177 serum samples tested by ELISA and 168 tested by PCR, 109 were positive for B19V IgM antibodies (61.6%) and 87 (51.8%) were positive for B19V DNA. The B19V IgM antibodies and DNA were detected in serum samples collected across the provinces of DRC. Most of the sera that were positive in both assays originated from Katanga, in the southeast of DRC (Table 1).

To determine the association of the proportion of B19V IgM with age, the serum samples were divided into three age groups: 9–12 months, 13–36 months, and 37–60 months. The frequency of B19V IgM antibodies was determined in each group and no significant difference was observed between the age groups ($p = 0.33$) (Table 2).

The proportion of samples positive for B19V IgM antibodies in males was 57.1% (52/91) and in females was 66.3% (57/86). There was no significant difference in proportions between the sexes ($p = 0.21$).

Discussion

Little is known about the epidemiology of parvovirus B19 in Sub-Saharan Africa, and there is anecdotal information about this virus in the paediatric population (Gabor et al., 2015; Duedu et al., 2013; Wildig et al., 2010). This study appears to be the first to investigate B19V as an aetiological cause of rash–fever illnesses in DRC. The present study detected the presence of B19V IgM in sera collected in the context of rash–fever syndromes among Congolese children less than 5 years old.
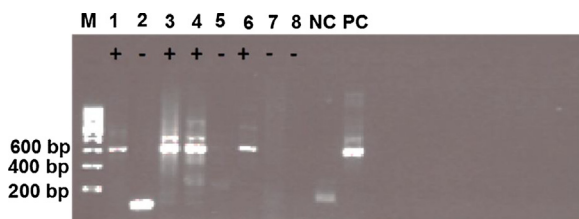
Figure 2. Agarose gel electrophoresis of PCR showing the 200-bp DNA ladder (M), DNA samples (lanes 1–8), negative control (NC) and positive control (PC) for B19V DNA.

Table 1
Human parvovirus B19 (B19V) DNA and IgM antibodies detected in sera from children <5 years of age with rash–fever illnesses in the Democratic Republic of the Congo.

Province	Number investigated	B19V DNA-positive ($n = 168$)	IgM-positive ($n = 177$)
Kinshasa	23	9	13
Bas-Congo	11	8	10
Bandundu	7	4	5
Orientale	17	10	12
Equateur	7	5	5
Katanga	53	22	27
East Kasai	30	15	19
West Kasai	9	4	4
Nord Kivu	5	2	2
Sud Kivu	6	3	5
Maniema	9	5	7
		87	109

Table 2
Proportion of human parvovirus B19 IgM antibodies in Congolese children under 5 years of age.

Age (months)	Parvovirus B19 IgM, n (%)		Total of sample
	Negative	Positive	
9–12	20 (33.9)	39 (66.1)	59
13–36	30 (45.5)	36 (54.5)	66
37–60	18 (34.6)	34 (65.4)	52
Total	68	109	177

The parvovirus B19 IgM antibodies and DNA were detected in serum samples from across the country, with most of the positive samples originating from Katanga Province. Indeed, according to data from the Ministry of Public Health, several outbreaks of measles-like rashes have occurred over the last 5 years in Katanga. As B19V IgM antibodies and DNA were found in sera from all provinces of DRC, it can be confirmed that B19V has spread countrywide, and this supports the hypothesis that B19V has a worldwide distribution (Rezaei et al., 2016; Zhang et al., 2016; Toshev et al., 2014; Tolfvenstam et al., 2000; Schwarz et al., 1989).

In this study, 61.6% (109/177) of sera were positive for B19V IgM antibodies and 51.8% (87/168) were positive for B19V DNA. One explanation for the discrepancy between the ELISA and PCR results might be the presence of B19V genotype 3, which is prevalent in Sub-Saharan Africa (Candotti et al., 2004; Hübschen et al., 2009; Corcoran et al., 2010). The detection limit for this genotype was low in this study due to mismatches of the primers used. Therefore, the investigation of B19V infection should include both ELISA and PCR. Moreover, the anti-B19V IgM antibodies and primers should be very specific and able to detect genotypes 1 and 3 of B19V.

The proportion of positive anti-B19V IgM in this study was surprisingly high (61.6%). In spite of the limited number of serum samples and the targeted group, the prevalence found in this study is within the range reported in previous studies conducted in Eritrea, Sao Tomé and Principe, Malawi, and mainland Mauritius (rates in the range of 56–91%) (Tolfvenstam et al., 2000; Schwarz et al., 1989).

In this study, there was no significant difference between age groups in children under 5 years of age. However, several studies involving populations with a wider age range have shown that the incidence of B19V infection increases significantly with age (Salimi et al., 2008; Röhrer et al., 2008). Unlike other studies, the sera selected and investigated in this study were limited to those from children <5 years of age. This situation may explain the absence of significant differences between the age groups.

The proportion of samples positive for anti-B19V IgM antibodies did not differ significantly between males and females in

this study. This observation is similar to that reported in a previous study conducted in Gabon (Gabor et al., 2015), while Bouafsoun et al. found that rash–fever illnesses in the North of Tunisia were more prevalent in females than males in the age range 19 months to 4 years (Bouafsoun et al., 2016).

In conclusion, this preliminary study demonstrated parvovirus B19 as an aetiological cause of rash–fever illnesses in children under 5 years of age suspected of measles and rubella in DRC. Although B19V receives little attention from health workers, local policy-makers, and the global health agencies in DRC, the present study shows that B19V should be considered in the laboratory investigation of rash–fever illnesses, particularly in the paediatric population. The results of this study will be helpful for the measles surveillance system, which aims to eradicate measles and rubella infection in DRC. Therefore, serological testing for B19V IgM should be included in the context of viral investigations of measles-like rashes as a routine blood test among children in DRC. Furthermore, there is a need to conduct further studies on a large cohort in order to determine the spatiotemporal pattern of B19V and to define the genotype(s) circulating in DRC.

Author contributions

TBW designed the study, performed laboratory and data analyses, and prepared the manuscript for publication. OMT participated in the data analysis and read the manuscript for publication. SMA and MNA read and critically revised the manuscript for publication. ESP participated in the field sample collection and performed the measles and rubella IgM tests. CK and JJTM were involved in designing the study, supervised the laboratory analyses, and read and critically revised the manuscript for publication. All authors have read and approved the final manuscript.

Ethical approval

The study was approved by the Ethics Committee of the School of Public Health, University Hospital of Kinshasa and the Ministry of Public Health in DRC. Serum samples and additional information were collected in the context of measles surveillance after obtaining verbal informed consent from each child's parents or legal guardians. The parvovirus B19 protocol was implemented as part of public health surveillance, as recommended by the DRC Ministry of Public Health. For confidentiality reasons, laboratory analyses were performed on anonymous samples and a unique code was used to identify the serum samples and corresponding data.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

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