

**PARVOVIRUS B19 FREQUENCY IN EXANTHEMATIC CASES INITIALY SUSPECTED AS
MEASLES OR RUBELLA INFECTIONS IN SÃO PAULO, BRAZIL**

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ABSTRACT

Parvovirus B19 infection may be misdiagnosed as measles, rubella, or other exanthematic diseases which are common in childhood. The clinical differential diagnosis for these infections can be misleading due to their similarity in most mild cases and the occurrence of atypical cases. In order to establish the occurrence of parvovirus B19 infection among children with exanthema, paired serum samples from 881 patients with presumptive diagnoses of measles or rubella were tested for IgM and IgG antibodies against parvovirus B19 through the ELISA capture method from 1993 to 1996, after being proved to be not measles or rubella infections. Parvovirus B 19 infection was confirmed in 80 individuals (9%) with IgM positive antibodies, and 240 (27%) with IgG positive antibodies. Sera positive for B19 corresponded to 1% of measles and 96% of rubella suspected cases. Therefore, parvovirus B19 should be considered for serological analysis when a child presents exanthema.

INTRODUCTION

Human parvovirus B19 (HHPV-B19) is a member of the *Parvoviridae* family, and belongs to the *Erythrovirus* genus, it is pathogenic to man and a common infection. It was discovered by accident by Cossart et al. (1975). The studies at the molecular, cellular, epidemiological, and clinical levels have been particularly fortunate insofar as the close relationship between laboratory developments and clinical observations is concerned. Until a few years ago, only research laboratories had the reagents or the motivation to perform B19 diagnostic assays, using conventional immunoassays for the anti-B19 IgG and IgM antibodies and molecular assay, such as PCR for B19 DNA. Unfortunately, variability in sensitivity and specificity, as well as the potential for specimen contamination, can make it difficult to interpret test results. A clear understanding of the advantages and pitfalls of diagnostic assays is important for evaluating B19 infection in individual patients, in reported disease associations, and in

epidemiological studies. Nowadays human parvovirus B19 is divided into three genotypes by phylogenetic analysis of partial sequences, combined with the erythrovirus sequences available in GenBank. The HPV B19 viruses correspond to genotype 1, whereas the V9 viruses are subdivided into genotypes 2 and 3, which have 10% nucleotide divergence. Whereas these genotypes generally cross-react serologically, PCR amplification may require specific primers. (Servant et al. 2002)

Measles, rubella, parvovirus B19 and other exanthematic diseases are common in children. The differential diagnosis is aided by the fact that the classic presentation of each disease is distinct. However, atypical presentations are not uncommon and can lead to misdiagnosis (Cubel et al. 1997, Oliveira et al. 2002, Valero 2006). The differential diagnosis may also be ambiguous because of incomplete information from family members, particularly if the patient presents a rash after the classic prodrome of the measles, rubella or parvovirus B19, which increases the difficulty of accurate diagnosis based on clinical symptoms. Another factor is the widespread measles and rubella vaccination program, which has resulted in the fact that many clinicians are unfamiliar with the signs of naturally occurring infections (Waldman 2007).

In order to establish the frequency of parvovirus B19 infection among children presenting exanthema, we carried out a serological study with serum samples from patients with presumptive diagnoses of measles or rubella, testing IgM and IgG antibodies for parvovirus B19.

MATERIAL AND METHODS

Serum samples.

Acute-phase and convalescent-phase sera were collected from 9,043 children and adults suspected of having measles or rubella from 1993 to 1996. This collection was part of the measles and rubella Surveillance Program in the state of Sao Paulo, Brazil. Serological assays for measles and

rubella were performed at the public health laboratory of the Adolfo Lutz Institute, in the city of Sao Paulo. The clinical diagnoses were based on generalized rash with more than 3-day-duration; fever (temperature $>38^{\circ}\text{C}$), and either cough, coryza or conjunctivitis.

Of these individuals, 1,153 proved to be rubella, and 107 measles infections. Paired sera from 881 patients, exonerative for rubella and measles, were available for further serological testing and they were screened for parvovirus B19 antibodies. Each of those samples was selected along with available information regarding patient age, date of onset of rash, and date of acute-phase and convalescent-phase serum collection. Acute-phase serum was collected when the patient presented an exanthema (mean day of collection, day 3 after onset of rash; range, 0-19 days), and convalescent-phase serum was collected an average of 11 days later (range, 1-54 days). All samples were collected after appropriate consent was obtained.

Enzyme-linked immunosorbent assay (EIA).

Serum samples were analyzed by indirect capture antibody EIA for specific IgG and IgM to detected human parvovirus B19 (Erdman et al. 1991) with baculovirus B19 capsid protein as the antigen (Kajigaya et al. 1989). IgM positive sera were retested after treatment to remove rheumatoid factor (Bond et al. 1986).

Polimerase chain reaction (PCR).

Oligonucleotide primers, specific to a conserved region of the NS1 gene coding for the B19 nonstructural protein, were prepared. Primers P1 and P6 were used for primary PCR amplification, yielding a 284-bp product; nested primers P2 and P5 were used to amplify an internal 102-bp product. The primer sequences, method of synthesis, and the standard PCR and nested-PCR assay have been previously described (Durigon et al. 1993).

RESULTS

B19 infection was confirmed by IgM and IgG seroconversion in 80 (9%) of the 881 selected cases, as shown in Table 1.

Table 1. IgM positive cases for measles, rubella and parvovirus B19 infection in 9,043 individuals from 1993 through 1996 in Sao Paulo, Brazil.

<i>Year</i>	<i>Measles</i>		<i>Rubella</i>		<i>B19</i>	
	<i>Samples</i>	<i>IgM</i>	<i>Samples</i>	<i>IgM</i>	<i>Samples</i>	<i>IgM</i>
1993	632	36(6%)	1579	251(16%)	69	10(15%)
1994	412	29(7%)	1556	334(21%)	233	30(13%)
1995	451	15(3%)	1632	467(29%)	263	21(8%)
1996	470	27(6%)	1430	101(7%)	316	19(6%)
Total	1965	107(5.4%)	6197	1153 (18.6%)	881	80(9%)

The suspected clinical diagnosis of those 881 cases were measles (8%), rubella (87%) and both measles or rubella (5%). Among the 80 cases IgM positive for B19, 1(1%) was mistaken for measles, 77(96%) were mistaken for rubella and 2 (3%) suspected to be rubella or measles.

All B19 IgM positive sera were also tested for seroconversion during the acute and the convalescent phase. In spite of being ELISA IgM positive, 11 sera were considered false-positive because reactivity for IgG was not observed either in the acute or in the convalescent sera for IgG. Furthermore, the PCR results for B19 were negative. The seroincidence in 80(9%) of the B19 IgM antibody positivity in 881 individuals, was distributed as follows: 7 (2%) out of 316 individuals, 0-2 years old; 8 (9%) out of 85 individuals, 3-4 years old; 32 (17%) out of 185 individuals, 5-10 years old;

7 (11%) out of 63 individuals, 11-15 years old; 3 (7%) out of 43 individuals 16-20 years old; 15 (13%) out of 119 individuals, 21-30 years old and 8 (11%) out of 70 individuals, over 30 years old (Figure 1).

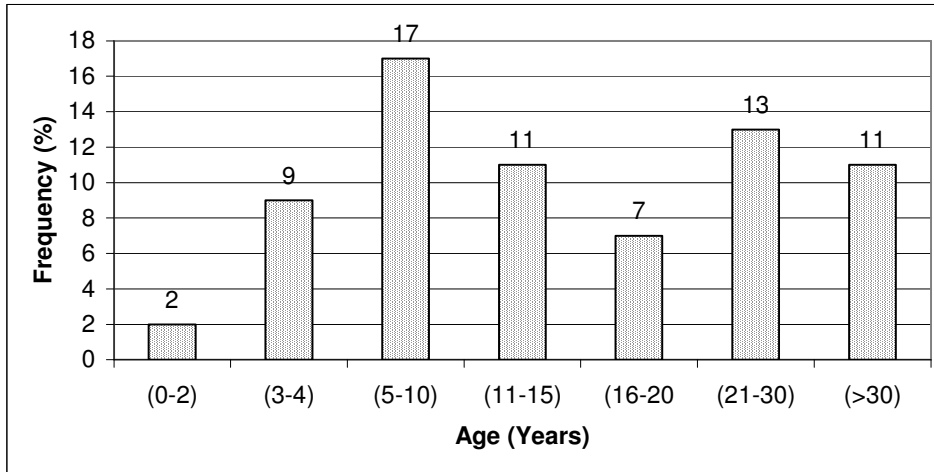


Figure 1. Age distribution of parvovirus B19 IgM positive patients.

The prevalence of B19 IgG antibodies increased with age. Out of 881 individuals, 240 (27%) cases of B19 IgG antibody positivity increased with age up to 10 years old as follows: 17 (5%) out of 316 individuals, 0-2 years old; 19 (22%) out of 85 individuals, 3-4 years old; 79 (43%) out of 185 individuals, 5-10 years old; 29 (46%) out of 63 individuals, 11-15 years old, 19 (44%) out of 43 individuals, 16-20 years old; 57 (48%) out of 119 individuals, 21-30 years old, and finally 20 (41%) out of 70 individuals, over 30 years old (Figure 2).

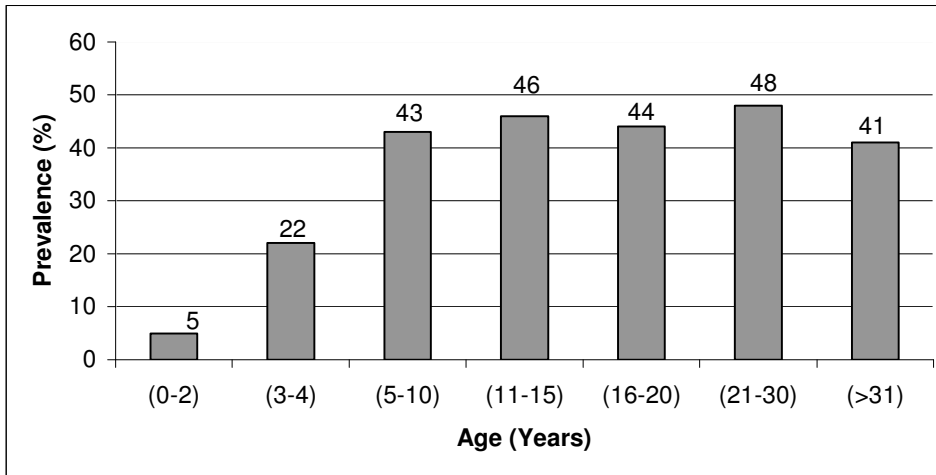


Figure 2. Age distribution of parvovirus B19 IgG positive patients.

The distribution of B19 infection of the 881 individuals showed that 245 (47%) women of reproductive age, defined as women from 10-50 years old (Al-Khan et al. 2003) demonstrated an incidence of B19 IgM antibodies: the maximum value was 17% in the 10-15 year old population, and of B19 IgG positive 28% in the 21-50 year old population (Figure 3).

The seasonal distribution of B19 infection from 1993 to 1995, for these 80 samples, the occurrence had a peak in the second half of each year, between late winter and the beginning of spring in Brazil, with sporadic cases of B19 throughout the year 1996 (Figure 4).

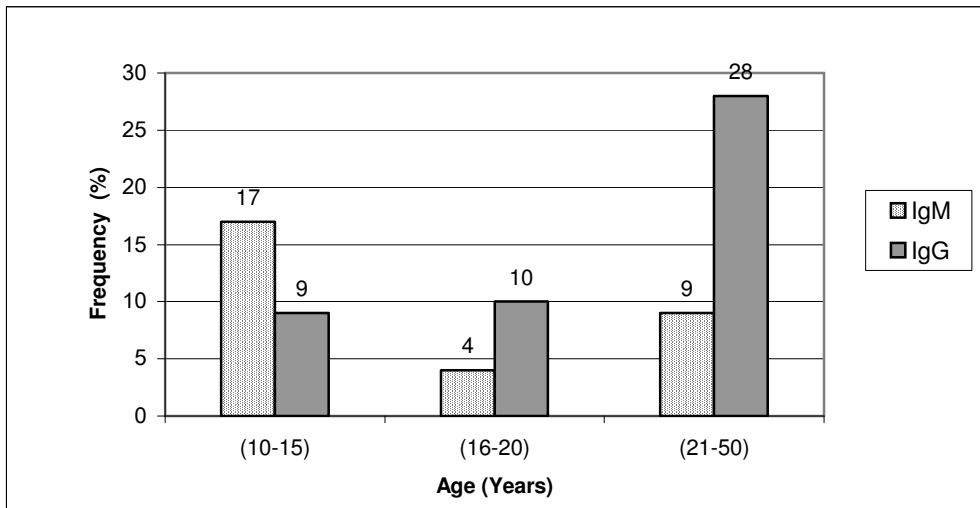


Figure 3. Frequency of parvovirus B19 IgM and IgG positivity in women of reproductive age (defined as 10-50 years).

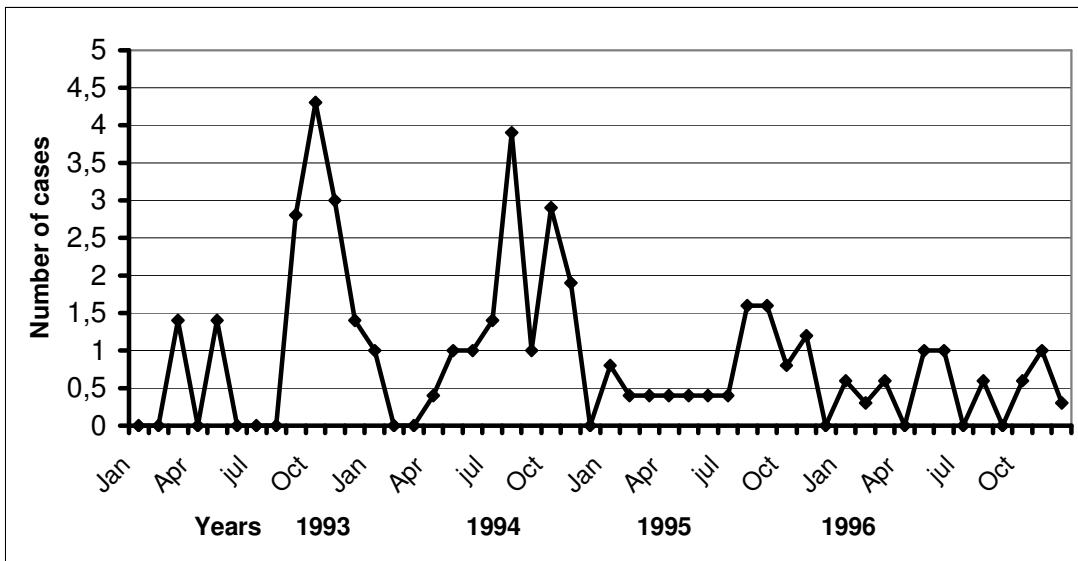


Figure 4. Seasonal distribution of parvovirus B19 infection from 1993 to 1996 in São Paulo, Brazil

DISCUSSION

In this study our data has shown that seroprevalence of parvovirus B19 infections increases with age. The seropositivity rate was 9% in the group under 5 years old, and the serological profile in this community also indicates that B19 infection rates are low before the age of five, being slow between

one and four years of age. This can be explained by apparently low exposure between susceptible and infected individuals in these age groups. The seroprevalence reach approximately 42% in the 5-31 age group, which results in high susceptibility during childbearing years with intense viral activity in the age groups, confirming an increasing trend in the proportion of seropositive individuals as age increases. This data is similar to other studies (Anderson 1987, Nascimento et al. 1990, Abarca et al. 2002, Ooi et al. 2002, Ziyaeyan et al. 2005, Wildig 2007, Huatuco et al. 2008).

The B19 IgM antibody showed different incidence with age, similar to other results reporters (Freitas et al. 1993, Ziyaeyan' et al. 2005). For B19, in the 5-10 year age group the incidence rate was 17%, higher than any other age group, which varied between 2% to 13%.

Women at reproductive age, from 10 to 50 years old (Al-Khan et al., 2003), presented a seroprevalence of 116(47%) IgG positive, and a seroincidence of 25(10%) IgM positive. The highest level of seroincidence 8 (17%) was at 10-15 years old. These results have shown the importance of new studies, in order to define how necessary is the immunization during reproductive age, because of the association of infection by parvovirus B19 with hydrops fetalis (Marton et al. 2005, Nymann et al. 2005, Ergaz 2006, Beigi 2008).

It is clear that parvovirus B19 infection occurs frequently in children less than two years of age in many continents included Brazil. For this reason, any preventive measures such as a vaccination will need to target the first year of life (Wildig 2007).

The seasonality of this study, during the period from 1993 to 1995, was similar to other studies, in other words, between late winter and spring (Cubel et al. 1996, Ramirez & Mastrobatista 2005), although in 1996 the incidence was lower than in previous years, and there was no late winter/spring peak.

Detection of outbreaks depends on the accurate diagnosis of a rash illness, Inaccurate diagnosis of such illness may result in disease spread, as well as inefficient use of limited resources, with shown by us in another studies at the Adolfo Lutz Institute and São Paulo University (Oliveira et al. 2002).

We are now reporting that 71 (8%) anti-B19 IgM positive cases were mistaken for measles, 769 (87%) for rubella and 41 (5%) for both rubella and measles. The proportionally high rate of misdiagnosing parvovirus B19 infection for rubella can be explained because parvovirus B19 disease is clinically more similar to rubella than to measles. Therefore parvovirus B19 should be included in the differential diagnosis of exanthematic diseases.

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