

HUMAN PARVOVIRUS B19 INFECTIONS AMONG EXANTHEMATIC DISEASES NOTIFIED AS MEASLES

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A total of 1397 sera collected from 1095 cases of exanthematic disease notified as measles in ES and RJ states during July 1992 to December 1994 were investigated. These sera were first tested for measles and rubella specific IgM. When they proved negative, they were tested for B19 specific IgM by an enzyme immunoassay. B19 infection was confirmed in 27 (2.5%) of these cases. Sera from 194 negative cases for measles and rubella IgM received from other Brazilian states were also investigated and B19 infection was confirmed for 11 of them. Sera from these 38 IgM positive cases for B19, were tested for anti-B19 IgG by an enzyme immunoassay and for B19 DNA by dot blot hybridization. Anti-B19 IgG antibodies were detected in most of the acute sera. B19 DNA was detected in the acute serum of one patient that had been splenectomized before. As the exanthem caused by human parvovirus infection may be clinically diagnosed as rubella, it could be important to diagnose B19 infection in Brazil since it is becoming prevalent as the cause of rash in countries where rubella is controlled by vaccination.

Key-words: Human parvovirus B19. Exanthematic disease. Measles. Rubella.

Parvovirus B19, first described as a chance finding in the serum of healthy blood donors⁸, is now known to cause a spectrum of disease in humans. The most common clinical presentation of B19 infection is the childhood exanthem, erythema infectiosum (fifth disease). This classically begins with a facial rash (slapped cheek) that spreads to the trunk and limbs where it takes on a reticulate or lace-like appearance^{3,20}. However the exanthem is highly variable, and may be misdiagnosed as rubella if laboratory investigation is not performed²². In Rio de Janeiro State, rubella and measles notifications are higher during the second half of the year^{5,21}. B19 infections were also reported in Rio de Janeiro for the same period^{10,11}.

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This work was partially supported by grants from COLAB/FNS, FAPERJ and CNPq, Brazil.

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Recebido para publicação em 17/01/96.

Since the National Measles Vaccination Campaign in Brazil, during which all children under 14 years old were vaccinated against measles (April to May, 1992), all patients presenting with rash, high fever, and at least one of the following symptoms: cough, coryza or conjunctivitis, should be investigated for evidence of measles infection⁵.

In this study all patients had initially been investigated for evidence of measles by detection of measles specific IgM. If this proved negative, their stored sera were tested for the presence of rubella specific IgM. As B19 virus has also been shown to cause exanthematic disease, we used all the negative sera for measles and rubella IgM, to search for evidence of recent human parvovirus B19 and study the occurrence of fifth disease in the states of Espírito Santo and Rio de Janeiro, Brazil.

MATERIAL AND METHODS

Patient's sera. Blood was taken by veinopuncture from cases notified as measles by health workers from the epidemiological surveillance in the States of Espírito Santo (ES) and Rio de Janeiro (RJ) for a 2 1/2-year period. The patient's sera had all been stored at -20°C until tested. Both acute and convalescent sera

were investigated if available. A total of 1397 sera collected from 1095 patients were examined for the occurrence of measles, rubella and B19 infection. Sporadic sera from 194 cases negative for measles and rubella IgM were also received from another Brazilian states (Alagoas, Bahia, Ceará, Distrito Fedral, Pará, Paraíba, Paraná, Pernambuco, Rio Grande do Norte and Rondônia). These cases were to search only for B19 infection.

Diagnosis of recent measles and rubella infection. Specific IgM detection of measles and rubella was done by using the commercial assays available at all laboratories that participate of Measles Control Program in Brazil: Enzygnost anti-measles IgM (Hoechst-Behring) for measles; and Rubenostika IgM (Organon Teknika BV) for rubella.

Diagnosis of human parvovirus B19 infection. Detection of specific anti-B19 IgM: All sera negative for measles and rubella IgM were tested for human parvovirus B19 specific IgM by an "in house" IgM capture enzyme immunoassay (MACEIA), as described previously⁹. For this test, serum containing 100, 33, 10, 3.3, 1.0 and 0.33 radioimmunoassay (RIA) arbitrary units (a.u.) of B19 IgM and normal human serum were used as positive and negative controls. Sera containing P/N values equal or greater than that one obtained with the 10 RIA a.u. control serum were considered as positive.

Detection of specific anti-B19 IgG: All anti-B19 IgM positive cases were tested for the presence of anti-B19 IgG antibodies by an "in house" enzyme immunoassay using recombinant B19 capsids produced in insect cells (Sf9) as antigen⁶. Briefly, flat bottomed microtiter plates (Immulon 2, Dynatech) were coated with 50µl of recombinant B19 capsids (1µg/ml; MedImmune Inc., USA) or 4% of SF9 cells diluted 1:1000 in 0.05M carbonate buffer (pH 9.6) and the plates were incubated for 1.5h at 37°C. The plates

were washed three times with PBS (pH 7.2) containing 0.05% Tween 20 (PBST), then 200µl of blocking buffer (PBS pH 7.2 plus 0.1% bovine serum albumine) was added, and the microtiter plates incubated for 1.5h at 37°C. After washing the plates, 50µl of patient serum diluted 1:20 in diluent buffer (blocking buffer plus 0.5% Tween 20) was added to wells in duplicate and the plates incubated for 1.5h at 37°C. After the wells were washed, 50µl of peroxidase-conjugate goat anti-human IgG (Sigma) diluted 1:20,000 in diluent buffer was added, and the plates incubated for 1h at 37°C. Finally, the plates were washed five times and incubated in the dark at room temperature with 100µl of substrate solution (tetramethylbenzidine in phosphate-citrate buffer). The reaction was stopped by the addition of 100µl of 2M H₂SO₄, and the absorbance at 450nm was measured with a Titertek Multiscan Plus spectrophotometer. Positive and negative B19 IgG sera were used as controls. The absorbance values obtained in the wells with the recombinant antigen (P) were compared to that one with the insect cells (N). Sera were considered as positive when P/N values were greater than 2.0 and the P - N values were greater than 0.10.

Detection of B19 DNA: All anti-B19 IgM positive cases were also tested for the presence of B19 DNA by "dot-blot" hybridization assay using a biotin-labelled probe¹⁷.

RESULTS

Sera were obtained from 1095 measles notified cases in RJ and ES States during July 1992 to December 1994, and tested for measles specific IgM. Measles infection was confirmed in only 52 (4.7%) cases (Table 1). About 40% of the cases were due to rubella infection, and relatively few B19 infections (27 cases - 2.5%)

Table 1 - Laboratory diagnosis among measles notified cases in Rio de Janeiro (RJ) and Espírito Santo (ES) States.

State	Year	IgM positive						IgM negative (all three)		Total
		Measles		Rubella		B19		n°	%	
		n°	%*	n°	%	n°	%	n°	%	
RJ	1992	29	9.6	131	43.4	4	1.3	138	45.7	302
	1993	13	3.3	168	43.2	5	1.3	203	52.2	389
	1994	2	1.1	74	42.3	11	6.3	88	50.3	175
ES	1992	3	4.6	25	38.5	3	4.6	34	52.3	65
	1993	4	2.9	71	51.8	3	2.2	59	43.1	137
	1994	1	3.7	7	25.9	1	3.7	18	66.7	27
Total		52	4.7	476	43.5	27	2.5	540	49.3	1095

* Percentage from the total number of cases.

were identified. However, among the cases received from ES, almost the same number of positives for measles and B19 infection were obtained. For RJ, during the period studied there was an increase in the incidence of B19 infection, while the number of cases due to measles infection was decreasing.

The seasonal distribution of B19 and rubella cases in ES and RJ States was examined (Figures 1 and 2). Rubella and B19 infection had a peak occurrence in the second half of the year, late winter and spring time; with sporadic cases of B19 and rubella during the first half of the year.

A total of 301 sera from 194 cases negative for measles and rubella IgM were obtained from other Brazilian states and investigated for B19 IgM. Twenty six cases were received from Pará (north Brazil) in the year of 1989 and B19 IgM could be detected in 5 (19.2%) cases. Among 47 cases from Paraná (south Brazil) in 1993, B19 infection was confirmed for 5 (10.6%) of these cases while for Distrito Federal we found only one positive (2.6%) in 38 cases in 1992.

From the 38 cases IgM positive for B19, for 19 of them acute and convalescent sera were available (Table 2). From 38 sera collected from

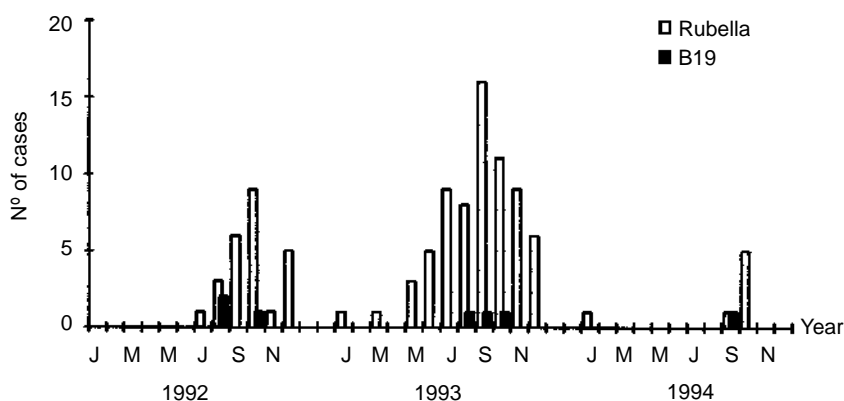


Figure 1 - Seasonal distribution of B19 and rubella cases in ES State.

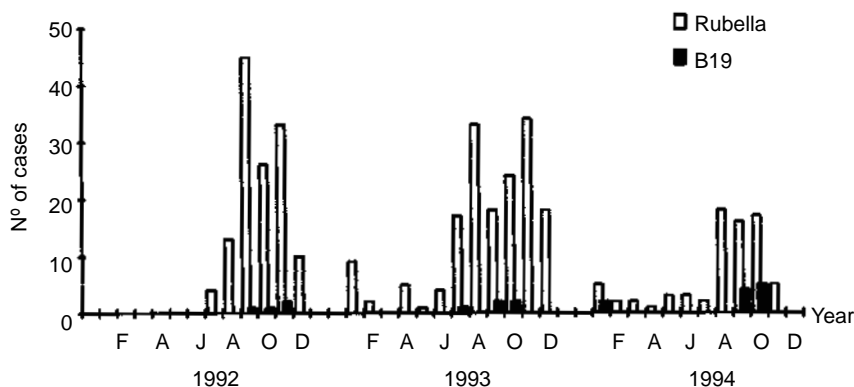


Figure 2 - Seasonal distribution of B19 and rubella cases in RJ State.

acute phase only two of them were negative for B19 IgM. B19 DNA could be detected in one of these IgM negative sera by dot blot hybridization. For this patient IgM and IgG was present only in the convalescent serum. B19 IgG was detected in 31 of the 35 acute sera tested and all but one convalescent sera was positive for B19 IgG. IgM and IgG antibodies for B19 virus could be detected in sera collected from 1 to 22 days after the beginning of the rash.

Table 2 - Anti - B19 IgG antibodies and B19 DNA testing for B19 IgM positive cases.

	B19 IgM		B19 IgG		B19 DNA		Total
	(+)	(-)	(+)	(-)	(+)	(-)	
Only acute serum	19	0	18	1	0	19	19
Acute and convalescent sera	17	2	13*	3	1**	18	19
Total	51	6	45	5	1	56	57

* For Pará acute sera from 3 patients and 4 convalescent sera were not available for IgG test.

** Acute serum from this patient was IgM and IgG negative. Convalescent sera was IgM and IgG positive.

DISCUSSION

Since the first association made by Anderson e cols¹ of an outbreak of erythema infectiosum (EI) and B19 infection many other reports have appeared and parvovirus B19 is now known to be the etiological agent of EI^{18 20}.

The first description of EI with laboratory confirmation in Brazil was done during 1987 in Belém, PA¹². Although anti-B19 IgM detection was possible only for three patients, similar clinical findings were reported for ten other cases during a four - year period, 1984 to 1987¹⁶. Most of these cases occurred during the first half of the year (January to June). During the following years (1988 to 1989) twenty-two laboratory confirmed cases were reported in Belém¹⁴. Another report from the same area for 1989 to 1990 found just one positive case out of 42¹³.

We are now reporting 27 (2.5%) anti-B19 IgM positive cases among 1095 cases reported as measles in RJ and ES states during the years 1992 to 1994. Shirley e cols²² detected B19 IgM positive sera in 6.8% of 627 cases of rubella like-illness in the West Midlands, UK, also during a 2 1/2 - year period (1983 to 1985). The proportion of positive cases found in RJ and ES States is lower and this can be easily explained because EI is clinically more similar to rubella than to

measles. In the West Midlands study the percentage varied from 3% to 19% for different years, and probably 1985 was an epidemic year for B19 in the UK¹. In our present study we probably lose an epidemic period.

It is difficult to discuss the results found for another Brazilian states because only for ES and RJ states a follow-up for a 2 1/2 - year period to investigate the occurrence of measles, rubella and B19 infections was done.

Our results for etiology of exanthematic disease in RJ and ES clearly show the impact of measles mass vaccination over the measles virus circulation in both States. From the 92 cases received from ES in the years of 1992 and 1994, we had the same number of positives for measles and B19. In a previous study of an outbreak of exanthem in Niterói, RJ during 1991 we obtained 80% of laboratory confirmation of 112 measles reported cases¹⁹. In that study no cases with anti-B19 IgM were found and rubella was diagnosed in only four cases.

Diagnosis of erythema infectiosum is mainly based on serology, because specific antibodies are present at the onset of the symptoms. At this time, in normal individuals viraemia can no more be detected². We were able to detect B19 DNA in a patient with rash. This patient had been splenectomized before and it may have contributed to the longer viraemia. Prolonged viraemia has been described to occur when immunocompromised patients are infected with B19²³.

In our study no relationship between days of disease and antibody-detection could be observed, since IgM and, in most cases, IgG were present after one day of the onset of the rash.

In about 50% of the cases the etiology of the rash was unknown. It is well known that erythematous rashes occur in many common viral infections, like arboviruses, infectious mononucleosis, enteroviruses and the more recently described human herpesvirus 6 (HHV-6). Preliminary studies of HHV-6 infection in north-east Brazil detected seroprevalence rates of 70% and children were found to possess high level of antibodies¹⁵. So the etiology of these cases remains to be elucidated.

Amplify this study would be desirable as also to consider B19 infection as an alternative diagnosis to rubella especially during pregnancy in which B19 virus can cross the placenta and

harms the fetus. Human parvovirus B19 infection is clinically indistinguishable from rubella²² and is becoming prevalent as the cause of rash in countries where rubella is controlled by vaccination⁷. It could be particularly important to diagnose B19 infections in Brazilian states like Distrito Federal, Paraná and São Paulo, in which rubella vaccination programs are in progress.

RESUMO

Um total de 1397 soros colhidos de 1095 casos de exantema notificados como sarampo, nos estados de ES e RJ, no período de julho de 1992 a dezembro de 1994, foram investigados. Estes soros foram inicialmente testados para sarampo e rubéola por detecção de IgM específica. Os casos negativos foram então testados para a presença de IgM específica anti-B19 por um ensaio imunoenzimático. A infecção pelo B19 foi confirmada em 27 (2,5%) destes casos. Soros de 194 casos negativos para sarampo e rubéola provenientes de outros estados brasileiros foram também investigados, e a infecção pelo B19 pode ser confirmada em 11 destes casos. Os soros dos 38 casos IgM positivos para B19 foram testados para a presença de IgG específica anti-B19 por um ensaio imunoenzimático, e para a presença do ADN viral por hibridização em "dots" (dot blot). IgG específica anti-B19 pode ser detectada na maioria dos soros de fase aguda, e o ADN viral foi detectado no soro de fase aguda de um paciente esplenectomizado. Como o exantema causado pela infecção pelo parvovírus humano pode ser clinicamente diagnosticado como rubéola, seria importante realizar o diagnóstico desta virose no Brasil, já que um aumento no número de casos de exantema por B19 tem sido relatado nos países onde a rubéola é controlada por vacinação.

Palavras-chaves: Parvovírus Humano B19. Doença Exantemática. Sarampo. Rubéola.

ACKNOWLEDGEMENTS

This work would not be possible without the aid of the responsables for Measles Surveillance in the states of Rio de Janeiro (Marcia Mello) and Espírito Santo (Alexandra M Ferreira). The authors wish to thank Ana CR Alferes, Monica N Mazorche, Verônica CR Soares and Zélia PA Ferro for technical support. RCN Cubel received a CNPq fellowship as part of her PhD studies at the Instituto de Microbiologia, Universidade Federal do Rio de Janeiro. The authors also wish to thank Dr. Kevin E Brown

(Hematology Branch, NIH, USA) for critical reading of the manuscript.

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