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Primary human herpesvirus-6 and -7 infections, often coinciding, misdiagnosed as measles in children from a tropical region of Brazil

S. A. OLIVEIRA[1], D. J. TURNER[2*], W. KNOWLES[3], J. P. NASCIMENTO[4†], D. W. G. BROWN[3] AND K. N. WARD[5‡]

[1] *Disciplina de Doenças Infecciosas e Parasitárias, Rua Marquês do Paraná, 303, 2o. andar, Niterói, Rio de Janeiro, Brazil 24030-210*
[2] *Department of Infectious Diseases, Imperial College School of Medicine, Hammersmith Campus, Du Cane Road, London W12 ONN, UK*
[3] *Enteric and Respiratory Virus Laboratory, Virus Reference Division, Public Health Laboratory Service, Central Public Health Laboratory, London NW9 5HT, UK*
[4] *Department of Virology, Instituto Oswaldo Cruz, Av Brasil 4365-Manguinhos, 21045 900 Rio de Janeiro, Brazil*
[5] *Department of Virology, Royal Free and University College Medical School, Windeyer Institute of Medical Sciences, 46 Cleveland Street, London W1T 4JF, UK*

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SUMMARY

We investigated primary human herpesvirus-6 and -7 (HHV-6, HHV-7) infections as a cause of rashes incorrectly diagnosed as measles in Brazilian children. Sera from 124 patients, aged 4 months to 17 years, from the states of Rio de Janeiro and Espírito Santo, in whom measles, rubella and parvovirus B19 infections had been excluded, were studied using indirect immunofluorescence antibody avidity tests; 38 (31%) had evidence of primary HHV-6 and/or HHV-7 infections. Twenty four children had primary HHV-6 infection, either recent or coincident with the rash, and similarly 31 had primary HHV-7 infection. Remarkably, almost half (17) of primary infections were dual HHV-6 and HHV-7 infections with the majority, 12 (71%), in children less than 1 year old. HHV-7 infection occurred earlier than previously reported, perhaps due to socioeconomic and tropical conditions in this region of Brazil, and thus coincided with the HHV-6 infections. This study also highlights the difficulties of diagnosing a rash illness on clinical grounds alone.

INTRODUCTION

Exanthem subitum, or *roseola infantum*, is a classical rash disease of early childhood in which there is a high fever of very abrupt onset lasting 3–4 days, and a maculopapular rash which appears as the child's temperature falls by crisis. However, despite this well defined syndrome it was noted many years ago that the rash is frequently misdiagnosed as that of either measles or rubella [1]. This clinical impression was finally confirmed in the laboratory when primary human herpesvirus-6 (HHV-6) infection, a recently proven cause of *exanthem subitum* [2], was shown to be commonly misdiagnosed as measles or rubella in British children under the age of 2 years [3]. *Exanthem subitum* is now known to be caused by both HHV-6 and the closely related human herpesvirus-7 (HHV-7) [4]. Any study of the aetiology of rashes misdiagnosed clinically as measles or rubella must therefore include diagnostic methods able to distinguish between

* Present address: Department of Infectious Diseases and Microbiology, Centre for Molecular Microbiology and Infection, Flowers Building, Imperial College London, South Kensington, London SW7 2AZ, UK.
† Present address: Biomanguinhos, Fundação Oswaldo Cruz, Av Brasil 4365-Manguinhos, 21045 900 Rio de Janeiro, Brazil.
‡ Author for correspondence.

primary HHV-6 and primary HHV-7 antibody responses. In this paper we describe the application of antibody avidity tests, which can differentiate primary HHV-6 from primary HHV-7 infection, and *vice versa* [5], to sera from patients with a presumptive diagnosis of measles but for whom there was no serological evidence of measles, rubella or human parvovirus B19 infection.

METHODS

Study population

In April and May 1992 the Brazilian Ministry of Health launched a countrywide mass immunization campaign against measles. After the campaign an intensive surveillance system was instituted and physicians were required to notify suspected cases. The clinical case definition was an illness characterized by fever $\geqslant 38{\cdot}3\ ^{\circ}C$, a generalized maculopapular rash of $\geqslant 3$ days duration, and at least one of the following: cough, coryza or conjunctivitis [6]. Acute and convalescent sera were collected from 1095 patients with suspected measles between 1992 and 1994 as part of the Measles Surveillance Programmes in the states of Rio de Janeiro and Espírito Santo. The sera were tested at the Brazilian National Measles Reference Centre for measles IgM by the method of Hummel et al. [7], rubella IgM using a commercial ELISA (Organon Teknika, Belgium) and human parvovirus IgM by the method of Cubel et al. [8]. Five percent (52) were IgM positive for measles, 43% (476) for rubella and 2·5% (27) for parvovirus B19. Sera from 11 individuals had also been tested for dengue virus IgM by the method of Gunasegaran et al. [9] and found to be negative. There was no diagnosis in 540 (49·5%) of cases.

Serum samples

Sera were chosen from children up to 17 years old for HHV-6 and HHV-7 testing if measles, rubella and parvovirus infections had been excluded by serological testing as described above, the patient's age, date of onset of rash, and date the serum was taken were all known and there was sufficient serum for testing.

Indirect immunofluorescence tests for HHV-6 and HHV-7 antibodies

HHV-6 and HHV-7 IgG were detected by immunofluorescence as described previously [5, 10]. Serum was tested in doubling dilutions starting at 1 in 10. Since there is limited cross-reactivity between HHV-6 and HHV-7 antibodies, titres of 40 or less were defined as negative [11].

Avidity of HHV-6 and HHV-7 IgG antibodies

The IgG tests described above were modified to elute low avidity antibody with urea [5, 12]. Sera with a titre of 80 or more were tested for antibody avidity and those samples whose antibody titre was reduced eight-fold or greater by urea were defined as low avidity and conversely sera whose titre was reduced four-fold or less were defined as having high avidity [12].

Definitions

Date of onset of illness was defined as the day of onset of rash. *Acute phase serum sample* was defined as a serum taken within 9 days of onset of rash. *Convalescent phase serum sample* was defined as a serum taken 10 days or later after onset of rash. *Primary HHV-6 or HHV-7 infection at the time of rash* was identified if paired acute and convalescent serum samples showed seroconversion of four-fold or greater to low avidity IgG antibody to either virus [5, 12].

Recent primary HHV-6 or HHV-7 infection of uncertain date of onset was identified where low avidity antibody was detected within the first 30 days after onset of rash [3] but there was no evidence of seroconversion to that particular virus.

Exclusion of primary HHV-6 or HHV-7 infection was accepted when a serum sample had high avidity antibody to that particular virus within the first 30 days after onset of rash [3], or a convalescent serum sample was negative for antibody to that particular virus.

Indeterminate primary HHV-6 or HHV-7 infection was when the serum available contained high avidity antibody to that particular virus but was taken 31 days or more after onset of rash [3], or the only serum available was acute phase and negative for antibody to that particular virus. In these cases, primary infection could not be excluded.

RESULTS

One hundred and sixty-four sera taken from 124 patients, aged 4 months to 17 years, were available for HHV-6 and HHV-7 antibody testing; there were 40 paired sera but only one serum available from the

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Table 1. *HHV-6 and HHV-7 IgG antibody avidity in relation to age*

	Serum HHV-6 IgG			Serum HHV-7 IgG			
Age (years)	High avidity no. (%)	Negative no. (%)	Low avidity no. (%)	High avidity no. (%)	Negative no. (%)	Low avidity no. (%)	No. sera tested
<1	10 (27)	13 (35)	14 (38)	9 (24)	15 (41)	13 (35)	37
1	13 (57)	5 (22)	5 (22)	7 (30)	6 (26)	10 (43)	23
2	15 (88)	2 (12)	0	13 (76)	1 (6)	3 (18)	17
3	6 (75)	2 (25)	0	8 (100)	0	0	8
4–9	17 (71)	3 (12·5)	4 (17)	19 (79)	1 (4)	4 (17)	24
10–17	13 (87)	1 (7)	1 (7)	13 (87)	1 (7)	1 (7)	15
Total	74 (60)	26 (21)	24 (19)	69 (56)	24 (19)	31 (25)	124

remaining 84 children. The 164 sera had been taken at a median of 9·5 days after onset of rash (range 1–52 days).

The results of HHV-6 and HHV-7 IgG antibody avidity tests for the 124 children are shown in relation to age in Table 1. In the 40 cases where paired samples were available the results of only one serum are given; these were from convalescent sera taken within 10–29 days of the rash except for three cases where the results of acute phase sera are given instead since no sample had been taken between 10 and 29 days. Overall, for the 124 sera the samples had been taken at a median of 11 days after onset of rash (range 1–46 days).

Sera with high avidity HHV-6 or HHV-7 IgG

The 74 sera containing high avidity HHV-6 IgG were taken at a median of 13 days after onset of rash (range 1–46 days); 33 were taken within 9 days, 39 between 10 and 29 days and the remaining 2 sera at 33 and 46 days, respectively. Primary HHV-6 infection could therefore be excluded in all but two of these cases. The geometric mean titre of the sera with high avidity HHV-6 IgG was 434 (95% confidence limits 311–607). Similarly the 69 sera containing high avidity HHV-7 IgG were taken at a median of 11 days after onset of rash (range 1–46 days); 26 were taken within 9 days, 42 between 10 and 29 days and the remaining serum at 46 days. Primary HHV-7 infection could therefore be excluded in all but one of these cases. The geometric mean titre of the sera with high avidity HHV-7 IgG was 441 (95% confidence limits 356–547). The lowest proportion of sera with high avidity IgG to HHV-6 or HHV-7 occurred in children less than 1 year old (27 and 24% respectively), an increasing proportion in children aged between 1 and 2 years (57 and 30% respectively) and the highest proportions in the subsequent years.

Sera negative for HHV-6 or HHV-7 IgG

The 26 sera negative for HHV-6 IgG were taken at a median of 7·5 days after onset of rash (range 1–28 days); primary HHV-6 infection could be excluded in the 12 cases where the serum was antibody negative 10 days or more after the rash. Similarly, the 24 sera negative for HHV-7 IgG were taken at a median of 9·5 days after onset of rash (range 1–33 days); primary HHV-7 infection could be excluded in the 12 cases where the serum was antibody negative 10 days or more after the rash. The highest proportion of HHV-6 or HHV-7 IgG negative sera was found in children less than 1 year old (35 and 41% respectively), with lower proportions in children aged between 1 and 2 years (22 and 26% respectively) and the lowest proportions in subsequent years.

Sera with low avidity HHV-6 or HHV-7 IgG

The 24 sera containing low avidity HHV-6 IgG were taken at a median of 15 days after onset of rash (range 7–25 days). In eight cases where there was also an acute sample, there was evidence of primary HHV-6 infection at the time of rash since seroconversion had been demonstrated by testing the two samples in parallel, but in the remaining 16 cases with no evidence of seroconversion the results indicated a recent primary infection of uncertain date of onset. The geometric mean titre of the sera with low avidity HHV-6 IgG was 1140 (95% confidence limits 655–1995) and the geometric mean reduction in titre by urea 21-fold (95% confidence limits 16–28).

Similarly, the 31 sera containing low avidity HHV-7 IgG were taken at a median of 13 days after onset of

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rash (range 1–25 days). In nine cases where there was also an acute sample, there was evidence of primary HHV-7 infection at the time of rash since seroconversion had been demonstrated by testing the two samples in parallel, but in the remaining 22 cases there was no evidence of seroconversion and the results therefore indicated a recent primary infection of uncertain date of onset. The geometric mean titre of the sera with low avidity HHV-7 IgG was 313 (95% confidence limits 203–483) and the geometric mean reduction in titre by urea 32-fold (95% confidence limits 23–45).

The highest proportions of sera with low avidity IgG to HHV-6 or HHV-7 occurred in children less than one year old (38 and 35% respectively) and in children aged between 1 and 2 years (22 and 43% respectively).

Table 2. *Low avidity IgG antibody to HHV-6 and/or HHV-7 in relation to age*

Age (years)	No. sera tested	No. sera with low avidity antibody* HHV-6 only	HHV-7 only	HHV-6 & HHV-7
<1	37	2†	1‡	12 (5)
1	23	2	7	3 (1)
2	17	0	3	0
3	8	0	0	0
4–9	24	3	3 (1‡)	1 (1)
10–17	15	0	0	1 (1)

* Numbers in brackets are the number of cases where there was evidence of primary infection at the time of rash as demonstrated by seroconversion between acute and convalescent sera.
† Serum negative for HHV-7 antibody.
‡ Serum negative for HHV-6 antibody.

Children with primary infection

Twenty-four (19%) of the 124 children had evidence of primary HHV-6 infection, either recent or coincident with the rash. Primary HHV-6 infection could be excluded in 84 (68%) of cases but in the remaining 16 (13%) it was not possible to confirm or exclude primary HHV-6 infection. Similarly, the number of children with evidence of primary HHV-7 infection, either recent or coincident with the rash was 31 (25%). Primary HHV-7 infection could be excluded in 80 (65%) of cases but in the remaining 13 (10%) it was not possible to confirm or exclude primary HHV-7 infection.

Pattern of HHV-6 and/or HHV-7 IgG of low avidity in the children's sera

Further details of the IgG to HHV-6 and/or -7 in the 38 children with low avidity antibody are shown in Table 2; it can be seen that 17 (45%) of the sera contained low avidity IgG to both HHV-6 and -7.

Of the 15 cases less than 1 year old with low avidity antibody to HHV-6 and/or -7, 12 (80%) had low avidity IgG to both HHV-6 and -7, and in five of these children there was proof of dual primary infection coincident with the rash as seroconversion had been demonstrated by testing in parallel with an acute sample. In the three remaining cases with low avidity antibody to HHV-6 or -7 only, there was no evidence of antibody to the other virus.

In contrast, of the 15 cases in children aged 1 and 2 years with low avidity antibody to HHV-6 and/or -7, only three (20%) had low avidity IgG to both HHV-6 and -7 and in one of these children there was proof of dual primary infection coincident with the rash. In the 10 cases with low avidity antibody to HHV-7 but not HHV-6, HHV-6 antibody of high avidity was detected, and likewise in the remaining two cases with low avidity antibody to HHV-6, high avidity antibody to HHV-7 was detected.

Of the seven cases in children aged 4–9 years with HHV-6 and/or -7 low avidity antibody, there was one case with proof of dual primary infection in a child aged 5 years, one primary HHV-7 infection in a child aged 4 years who was negative for antibody to HHV-6, three cases with low avidity IgG to HHV-6 but high avidity antibody to HHV-7, and two cases with low avidity IgG to HHV-7 but high avidity antibody to HHV-6. In children aged between 10 and 17 years there was only one case with HHV-6 and/or -7 low avidity antibody; this was a child aged 14 years with dual primary infection.

DISCUSSION

The present study was designed to test sera from Brazilian children with a clinical diagnosis of measles, but without serological evidence of measles, rubella or parvovirus B19 infection, for HHV-6 and -7 antibody. We hypothesized that the true diagnosis in at least some of the cases might be *exanthem subitum* due either to primary HHV-6 infection or primary HHV-7 infection or indeed both. Although it has long been established that primary HHV-6 infection causes

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exanthem subitum [2], it is less clear how often primary HHV-7 infection on its own causes *exanthem subitum* [4]. Cases of primary HHV-7 infection usually occur in children known to be previously infected with HHV-6 [4, 13] and it has been shown that HHV-7 can reactivate HHV-6 *in vitro* [14]. It has therefore been suggested that in primary HHV-7 infection reactivation of pre-existing HHV-6 may be the true cause of the symptoms of *exanthem subitum*. However, there are occasional reports of primary HHV-7 infection-related *exanthem subitum* in children with no evidence of prior HHV-6 infection [4, 15]. It was anticipated that our investigation of the separate relationships of HHV-6 and -7 primary infections to *exanthem subitum* in the Brazilian children might shed light on this uncertainty regarding the role of HHV-7 alone.

Exanthem subitum caused by either primary HHV-6 or -7 infection is best diagnosed using indirect immunofluorescence tests for IgG antibody avidity to HHV-6 and HHV-7 [5, 12]. Primary infections may be diagnosed by the use of antibody avidity tests since antibody avidity increases progressively with time after exposure to an immunogen [16]. If antibody avidity is low this confirms recent primary infection, but if the avidity is high, primary infection must have occurred in the more distant past. Tests for antibody avidity have been applied successfully to sera for the diagnosis of many different human virus infections (for reviews, see [17, 18]) and rely on the fact that an agent which denatures protein will disrupt the antigen–antibody reaction preferentially, affecting low avidity but not high avidity antibody [19, 20]. In the case of the HHV-6 and -7 antibody avidity tests developed by Ward et al. [5, 12], the denaturing agent was urea and low avidity antibody could be reliably detected within 30 days of primary infection [3]. Applying these avidity tests to the Brazilian children's sera, almost all of which had been taken within 30 days of onset of rash, it was possible to identify recent primary infection even if there was only a single serum available from the child. If paired acute and convalescent sera were available and showed seroconversion to low avidity antibody then primary infection coincident with the onset of rash could be confirmed. Finally, primary infection with both HHV-6 and -7 could be deduced if low avidity antibody was present for both viruses [5]. Conversely primary infection to either of the viruses could be excluded if a serum had high avidity antibody within 30 days after onset of rash or if a serum sample was negative for antibody 10 days or later after onset of rash.

Turning to the results of HHV-6 IgG antibody avidity testing of the Brazilian children's sera with no previously demonstrated cause for their exanthematous illness, 19% had evidence of recent primary HHV-6 infection and the highest proportion (38%) was in children under 1 year old. Another study of children with rashes conducted in Niterói, Brazil, showed a similar proportion of 31% [21]. These proportions are comparable to that reported in a study of British children up to 2 years old with rashes [3]. Moreover in our study of Brazilian children about a third of the cases of primary HHV-6 infection were coincident with the rash as indicated by seroconversion thus confirming *exanthem subitum*; in one case of seroconversion the child was 14 years old at the time of the rash. Black et al. [22] used seroconversion to HHV-6 antibody measured by immunoblotting [23] as an indication of primary infection in a study of children with rashes misdiagnosed as measles or rubella and found results similar to ours except that no child seroconverted to HHV-6 above the age of 5 years.

Similarly, for the results of HHV-7 antibody avidity testing of the Brazilian children's sera studied here, 25% of the children had evidence of primary HHV-7 infection, the highest proportions of 35 and 43% being in children aged below 1 year, and 1 year old respectively although 5 cases occurred in children aged 4 years or older. About a third of these cases of primary HHV-7 infection were coincident with the rash thus confirming *exanthem subitum*. These results are surprising as first infection with HHV-7 usually occurs later than with HHV-6, the median ages being 26 and 9 months, respectively [13]. Equally surprising is the finding in our study that 45% of the children with low avidity antibody had low avidity IgG to both HHV-6 and HHV-7 and there were eight dual seroconversions suggesting *exanthem subitum* caused by either HHV-6 or HHV-7 or both. In contrast, only 5/54 (9%) of sera with low avidity IgG contained such antibody to both HHV-6 and -7 in a survey of 269 sera from British children taken for routine diagnosis of various viral infections [5] and dual primary HHV-6 and -7 infection as confirmed by dual seroconversion has only rarely been reported in the literature. Ward et al. [5] described two British children who seroconverted with low avidity antibodies to both viruses, and in each case the child was proven to be infected with both viruses as HHV-6 and -7 DNAs were found in various samples. Black et al. [22] diagnosed four cases of dual primary infection by seroconversion

in Brazilian children from the state of São Paulo, Brazil.

The explanation for the high proportion of children in our study with low avidity antibody to both HHV-6 and HHV-7 is very unlikely to lie in antibody cross-reactivity between the viruses as measured by indirect immunofluorescence tests. This method is the accepted gold standard for HHV-6 and -7 testing and as already noted dual low avidity antibody has only rarely been detected. Moreover, the limited cross-reactivity between naturally-induced human antibodies against the two viruses [11, 23, 24] had been taken into account by defining all antibody titres of 40 or less to either virus as negative. In fact, the geometric mean titres of the sera with low avidity antibodies to HHV-6 or -7 were both at least eight-fold higher than the cut-off titre of 40 and there was no doubt that the antibody was low avidity since the geometric mean reductions in titre in the presence of urea were 21-fold and 32-fold respectively. It therefore must be concluded that in the case of a serum with dual low antibody avidity primary infection with both HHV-6 and -7 has recently occurred. Similarly, in the case of dual low avidity antibody seroconversion coincident with the rash, the child should be diagnosed with *exanthem subitum*.

The much higher proportion of children with dual HHV-6 and HHV-7 primary infections from the Brazilian states of Rio de Janeiro and Espírito Santo in our study as opposed to those reported by Black et al. [22] in the Brazilian state of São Paulo could be explained by differences in antibody testing methodology but is much more likely to be due to the fact that our results for both HHV-6 and -7 concern, for the first time, a population in the tropics. It is also very likely that the socioeconomic conditions, particularly as regards residents in favellas of a tropical region of Brazil, have influenced the results showing that primary HHV-7 infections occur somewhat earlier than in other parts of the world thus coinciding with primary HHV-6 infections and giving rise to the high incidence of dual primary infections.

Finally, our study has confirmed that primary HHV-6 infection, dual primary HHV-6 and -7 infection, primary HHV-7 infection in those already infected with HHV-6 and very rarely, primary HHV-7 infection alone, can all cause *exanthem subitum* which is frequently misdiagnosed as measles. Furthermore we have found that very occasionally both primary HHV-6 and HHV-7 infections can cause *exanthem subitum* in children older than 5 years. In this context, a delayed primary HHV-7 infection with serious neurological complications in an adult in the UK has recently been reported [25].

The present findings that many of the children misdiagnosed with measles in fact had an *exanthem* due to HHV-6 and/or -7 is of importance for measles surveillance programmes in both temperate and tropical regions since it clearly shows that these *exanthemata* cannot be reliably diagnosed clinically and require reference laboratory confirmation with suitable specialized tests.

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