ORIGINAL ARTICLE

Causes of morbilliform rash in a highly immunised English population

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Aims: To determine the causes of morbilliform rash and fever in a population with high vaccination coverage for measles and rubella.
Methods: Comprehensive laboratory investigation additional to routine oral fluid testing of children presenting to primary care physicians in East Anglia, England.
Results: Laboratory confirmation of infection was obtained in 93 (48%) of 195 children: parvovirus B19 in 34 (17%); group A streptococcus in 30 (15%); human herpesvirus type 6 in 11 (6%); enterovirus in nine (5%); adenovirus in seven (4%); and group C streptococcus in six (3%) (four individuals tested positive for two agents). None had measles or rubella.
Conclusions: Oral fluid testing to cover infections additional to measles and rubella aids clinical management and is likely to maintain uptake of testing, which is essential for measles and rubella surveillance in highly immunised low incidence populations.

The infectious causes of morbilliform rash and fever in childhood are varied and include measles virus, rubella virus, group A streptococci (GAS)—the cause of scarlet fever, parvovirus B19, non-polio enteroviruses, adenoviruses, and human herpesvirus type 6 (HHV6).[1] Laboratory investigation is therefore necessary for accurate diagnosis. In populations with high levels of immunisation against measles and rubella, other causes of rash-fever illness predominate, but knowledge of the epidemiology and natural history of these infections is generally limited.[2 3]

Oral fluid (saliva) testing for measles and rubella antibody was introduced in England and Wales following the 1994 vaccination campaign.[4–6] The test is non-invasive and reliable and has proved popular with doctors investigating morbilliform rash-fever illness. The vast majority of suspected measles and rubella cases which are tested are not confirmed. It is therefore desirable to extend oral fluid testing to the other common causes of rash-fever illness so that positive diagnostic information can be provided in a higher proportion of cases. This would probably make oral fluid testing more attractive to physicians and carers and ensure continued high levels of laboratory surveillance for measles and rubella, which is crucial for their control and elimination, especially when the incidence of both diseases is low.[7 8]

In order to investigate the current causes of morbilliform rash and fever in a highly vaccinated population of English children, and to inform development of extended oral fluid testing, children presenting to general practitioners with morbilliform rash and fever underwent comprehensive laboratory investigation at the same time as routine oral fluid testing for measles and rubella.

SUBJECTS AND METHODS

Subjects were children under 16 years of age presenting with a morbilliform rash and fever to participating general practitioners (primary care physicians) in the East Anglian region of England between 1996 and 1998, and whose parent or guardian gave informed consent to participation.

Ethical approval

Ethical approval was obtained from health authority local medical research ethical committees.

Recruitment and liaison with general practices

Consultants in communicable disease control wrote to general practitioners within their health authorities inviting them to participate. Practices expressing interest received a preliminary visit from the study nurse, who explained the protocol in detail and sought participation. Participating practices were asked to recruit up to 10 children each and were provided with comprehensive written instructions and materials for documentation and the taking and dispatching of specimens. A blood spot and oral fluid sample were taken for virology, and throat swabs for virology and bacteriology from each child. Practice staff instructed parents and guardians to obtain and submit a stool sample and a further oral fluid swab from the child at home, and provided written instructions and materials for collecting these samples. The study nurse stayed in regular contact with participating practices, and could be contacted by parents and guardians for further advice and assistance during the study.

Microbiological investigation and interpretation

Each child had the following samples taken for microbiological investigation.

- At presentation in the general practice surgery:
  (1) Oral fluid swab for measles and rubella IgM.
  (2) Blood spot for parvovirus B19 IgM and HHV6 collected by finger prick using a Tenderlett atraumatic skin incision device (International Technidyne Corporation, Edison, NJ, USA) and spotted on to filter paper.
  (3) Throat swabs for routine bacterial and viral culture. Two cotton wool throat swabs were passed in tandem over the tonsils, the first placed in bacteriology and the second in virology transport medium.
- At home: seven days after the onset of rash-fever illness:
  (4) Stool for routine viral culture.
  (5) Second oral fluid swab for measles and rubella IgM.

Abbreviations: GAS, group A streptococci; GCS, group C streptococci; HHV, human herpesvirus; PCR, polymerase chain reaction

Table 1 Number (%) of laboratory confirmed infections in cases of rash-fever illness by age group

	Throat isolate		Viral culture		Parvovirus B19	HHV6		
Age group	Group A streptococcus	Group C streptococcus	Adenovirus	Enterovirus	IgM positive	PCR or LAA	Any*	Total
Under 1 y	0 (0)	1 (3)	1 (3)	2 (6)	1 (3)	7 (19)	12 (33)	36
1 y	2 (4)	1 (2)	2 (4)	5 (11)	2 (4)	4 (9)	16 (36)	45
2–3 y	13 (27)	0 (0)	3 (6)	1 (2)	12 (24)	–	28 (57)	49
5–9 y	14 (30)	3 (7)	1 (2)	1 (2)	13 (28)	–	29 (63)	46
10–15 y	1 (5)	1 (5)	0 (0)	0 (0)	6 (32)	–	8 (42)	19
Total	30 (15)	6 (3)	7 (4)	9 (5)	34 (17)	11 (6)	93 (48)	195

*Tested positive to one or more agents.
LAA, low avidity antibody.

Table 2 Number (%) GAS cases in the study population and cases of scarlet fever notified in England and Wales, 1996 to 1998

Age group	Confirmed GAS		Notifications of scarlet fever (21)	
Under 1 y	0	(0)	252	(2)
1–4 y	14	(50)	4351	(42)
5–9 y	13	(46)	4078	(40)
10–14 y	0	(0)	826	(8)
15–19 y	1	(4)	744	(7)
Total	28	(100)	10251	(100)

Culture of viruses from faeces

Approximately 0.5 g faeces was emulsified in 4 ml faecal extract medium, centrifuged at 1200 rpm for four minutes, and 0.2 ml was inoculated on to Hep-2, monkey kidney, and PLC-PRF5 cell cultures. Tubes were incubated at 37°C on rollers for 12 days and examined for the development of cytopathic effect. Whenever possible, viruses were typed by neutralisation with specific antisera.

Culture of viruses from throat swabs

Throat swabs in virus transport medium were vortexed and 0.2 ml was inoculated on to MRC-5, Hep-2, monkey kidney, and PLC-PRF-5 cell cultures. Tubes were incubated at 33°C for 14 days on rollers and examined for the development of cytopathic effect. Whenever possible, viruses were typed by neutralisation with specific antisera. Cells were also tested for haemadsorption every two days, using human O red cells. Children who had both stool and throat samples negative on viral culture were considered to have recent adenovirus and enterovirus excluded.

Culture of throat swabs for bacteria

Throat swabs were inoculated on to blood agar and incubated anaerobically for 24 hours. They were also inoculated on to Hoyle's agar and incubated at 37°C. Plates were examined, and isolates were identified by latex antibody agglutination and by biochemical tests using the API rapid ID 32 Strep kit.[9] Children with rash and fever whose throat swabs grew group A streptococcus were considered to have scarlet fever.

Oral fluid antibody testing for measles and rubella

Oral fluid samples were eluted and tested for measles or rubella specific IgM by antibody capture radioimmunoassay.[6 10]

Blood spot antibody testing for parvovirus B19 infection

Blood from a 0.5 × 0.5 cm area on which blood had been spotted was eluted by immersion for a minimum of two hours in 200 µl phosphate buffered saline. The eluate was considered to represent a 1/16 dilution of serum and was tested for parvovirus B19 IgM by antibody capture radioimmunoassay.[11]

Testing for human herpesvirus type 6 (HHV6) in children under 2

Blood spots from children under 2 years of age were tested for HHV6 antibody where sufficient eluate remained after testing for parvovirus B19 infection. Antibody was measured by an IgG avidity method using indirect immunofluorescence[12]; if the titre was reduced eightfold or more by the presence of 8M urea, the antibody was regarded as low avidity. Results were recorded as negative, low avidity, or high avidity. As reconstituted blood spots were equivalent to a serum dilution of 1/16, only samples with an antibody titre of ⩾1/64 could be taken to be low avidity; those with a lower titre, which was reduced by 8M urea were recorded as low titre. Polymerase chain reaction (PCR) for HHV6 was performed on 40 oral fluid samples. These samples included first samples from children under 2 years of age with negative or low titre antibody where sufficient oral fluid remained after testing for measles and rubella. DNA was extracted from the samples[13] and amplified in a nested PCR reaction using primers differentiating between HHV6 variants.[14] A child was defined as having evidence of recent HHV6 infection if serum was positive for low avidity antibody, or if oral fluid was PCR positive for HHV6 DNA combined with a negative or low titre antibody result. A child was defined as having recent HHV6 excluded if found to be positive for high avidity antibody (consistent with past infection) or antibody negative and PCR negative (in those with low titre antibody, recent infection could not be excluded).

RESULTS

A total of 195 children registered with 82 different general practitioners were recruited from 39 general practices during 1996 to 1998. General practices recruited between 1 and 39 (median 3) children. Only four practices recruited 10 children; two of these agreed to continue recruitment and had recruited 27 and 39 children, respectively, by the end of the study. A full set of samples was available for 155 (79%) of the 195 children. The most common sample missing was the stool sample (n = 23), although seven throat swabs for virology, eight for bacteriology (including one child who also had a missing stool specimen), and four blood spots were not submitted.

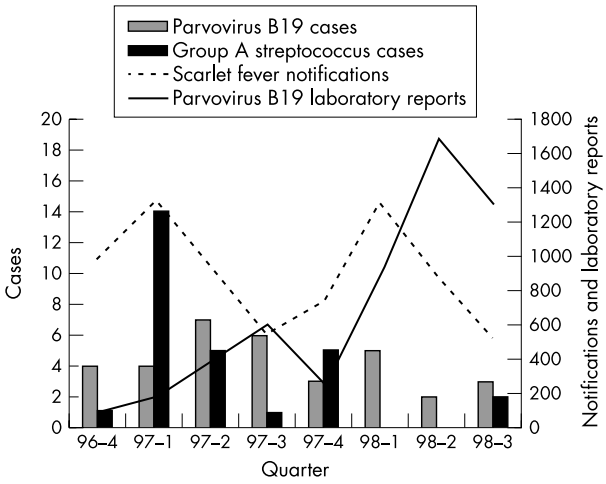

Figure 1 Number of study cases of parvovirus B19 and GAS by date of onset, parvovirus B19 laboratory reports, and scarlet fever notifications by quarter, 1996–4 to 1998–3.

No children had evidence of recent measles or rubella infection. Infection with one or more agents was confirmed in 93 (48%) of the 195 cases recruited (table 1). No evidence of infection was obtained for 102 (52%) of cases: this included 49 (43 per cent) of 114 cases aged 2–15 years and 53 (65 per cent) of 81 cases under 2 years of age in which HHV6 testing was also done.

The majority of children with confirmed infections had parvovirus B19 or GAS infection. Two children were positive for both infections. The proportion of cases with either infection was higher among school age than preschool children (table 1). The proportion of confirmed cases of parvovirus B19 in the older age group (10–15 years) was higher than the proportion with GAS.

The age breakdown of GAS infections reflected the age distribution of cases of scarlet fever notified nationally in 1996–98 (table 2). The age distribution of confirmed parvovirus B19 reports from laboratories does not reflect the true incidence of this infection in the community because serum testing is routinely performed only in people at risk of complications (for example, pregnant women).

Figure 1 shows dates of onset for cases with confirmed parvovirus B19 infection and GAS infection. The seasonality of GAS infections corresponded to the seasonality of national data on scarlet fever notifications. The seasonal trends in parvovirus B19 infection were less well correlated with national data on laboratory confirmed infections with parvovirus B19, but contrasted with the trends in GAS infections and scarlet fever notifications.

Documented fever, cough, coryza, and conjunctivitis were non-discriminatory and were broadly similar for each group of confirmed infections (table 3). Cough with coryza and conjunctivitis was documented in two cases of GAS and a single case of parvovirus B19 infection. Sore throat was reported in six (20%) cases of GAS infection but not in parvovirus B19 infection.

A small number of enterovirus, adenovirus, and group C streptococcal (GCS) infections were identified. One child had GCS and enterovirus (echovirus type 3) grown from throat swabs. The cases with GCS isolated were similar to those with GAS infection isolates: four (67%) of the six had documented fever, four (67%) had a cough/coryza, and two (33%) had a sore throat.

Adenovirus type 1 was isolated from four children, type 2 from two and type 3 from one child. One of the children with adenovirus infection also had GAS isolated from a throat swab. Six (86%) of the seven children with adenovirus infection reported cough, five (71%) had documented fever, and only one (15%) had conjunctivitis. No other features were reported.

A variety of enteroviruses were grown; the commonest single type was coxsackie virus A21, which was isolated from three children. Other strains included echoviruses (types 3, 9, and 18), a vaccine strain poliovirus type 1, and an untypeable enterovirus which were each grown from one child. Six (67%) of nine children with enterovirus infection had a fever, four (44%) had a cough, and one (11%) had conjunctivitis.

Of the children who underwent a full set of tests for HHV6, recent infection was confirmed in 7/27 (26%) less than 1 year of age and 4/29 (14%) of those aged 1 year. Eight of the 11 children had a fever, and three had diarrhoea, with or without vomiting.

Two children had other infections identified in addition to those outlined above. One infant had *Streptococcus pneumoniae* isolated (this child was also positive for recent HHV6 infection) and another child had RSV grown on viral culture (this child was also positive for recent parvovirus B19 infection).

DISCUSSION

In the post-vaccine era, where measles and rubella infection have become rare, the clinical diagnosis of rash-fever illness becomes more difficult. This has been illustrated by the low proportion of cases notified as measles and rubella, which can be confirmed by laboratory investigation.[8 15]

Other illnesses, which cause rash and fever, can be mistaken for measles and/or rubella and cause unnecessary anxiety or initiate inappropriate public health action. In addition, the diagnosis of measles or rubella in a fully vaccinated child could cause loss of confidence in the vaccination programme. For this reason, laboratory investigation of a high proportion of suspected cases should be sought. To maintain the motivation of practitioners in obtaining samples from such cases, however, providing an alternative diagnosis is desirable. This

Table 3 Symptoms associated with infections; number (%) of confirmed infections

	Confirmed infection											
	Parvovirus B19		GAS		HHV6		Enterovirus		Adenovirus		GCS	
Symptoms	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Documented fever	22	(65)	28	(93)	9	(82)	6	(67)	6	(86)	4	(67)
Cough	16	(47)	14	(47)	6	(55)	4	(44)	7	(100)	4	(67)
Coryza	16	(47)	14	(47)	0	(0)	1	(11)	0	(0)	1	(17)
Conjunctivitis	5	(15)	3	(10)	1	(9)	1	(11)	1	(14)	0	(0)
Cough, coryza, and conjunctivitis	1	(3)	2	(7)	0	(0)	0	(0)	0	(0)	0	(0)
Sore throat	0	(0)	6	(20)	1	f(9)	1	(11)	0	(0)	2	(33)
Total	34		30		11		9		7		6	

GAS, group A streptococci; HHV6, human herpesvirus type 6; GCS, group C streptococci .

study sought to obtain alternative diagnoses for cases of rash-fever illness occurring in the community. This will enable the development of appropriate investigation algorithms and facilitate the appropriate management of such children in the future.

In this study, no cases of measles or rubella were identified. This is probably a result of the consistently high MMR vaccine coverage (above 90%) in the Eastern National Health Service region since 1988. Many children presenting with rash-fever illness have evidence of either GAS infection or parvovirus B19 infection. GAS infection is usually associated with a sore throat but can cause scarlet fever, particularly in older children. Although carriage of GAS can occur, the similar seasonality and age distribution of GAS infections with scarlet fever notifications (table 2) suggests that this infection may be responsible for the rash illness. Parvovirus B19 infection is thought to be asymptomatic in up to 50% of cases, but up to 30% of infections are associated with a rash. GAS and parvovirus B19 were responsible for a high proportion of rash-fever illnesses in children aged 5–9 years (table 1).

Additional testing for HHV6 infection was performed in children under 2 years of age. This age group was chosen because primary HHV6 infection causes roseola infantum in young children and by 2 years of age most children have already been infected.[16] Recent HHV6 infections were identified in 11 (14%) of 81 children under 2.

Other than rash-fever, there were no specific clinical features consistently associated with GAS, HHV6, or parvovirus B19 infection. Both GAS and parvovirus B19 infection were commonly associated with cough, and a sore throat was reported in one fifth of GAS infections. More significantly, in terms of exclusion of measles, only a very small proportion of children had conjunctivitis. Data from national surveillance indicate that, in comparison, 93% of cases of confirmed measles have conjunctivitis in addition to either cough or coryza accompanying the rash-fever illness.[8]

It is unclear whether the less commonly identified infections were the cause of the rash-fever illness, as no healthy controls were investigated. Nasopharyngeal carriage of streptococci is fairly common and therefore the significance of the isolates of group C streptococcus and *Streptococcus pneumoniae* are unclear. Certain enteroviruses (echo 7, 9, 16, coxsackie A4, A5, A16) are known to be associated with a rash, but it is difficult to attribute the rash illness to all of the enterovirus infections identified in this study; at least one enterovirus identified was a polio strain in a recently vaccinated child. Adenoviruses have only rarely been associated with rashes and asymptomatic adenovirus infection is well documented.[17 18] The identification of RSV infection in one child is also of doubtful significance as the cause of the rash.

The highest proportion of children with no infection identified was in infants under 1 year. In this age group, testing for HHV6 was performed, but some infections may have been missed because insufficient specimen was available or because the blood spot was taken too soon after onset.

This study has identified two main alternative causes of rash-fever illness in childhood and a third candidate for rash-fever illness in infants and toddlers. The findings agree with those from a study in Finland where 993 children who had serum submitted for investigation of measles or rubella were investigated for parvovirus B19, enterovirus, HHV6, and adenovirus infections.[19] A possible aetiology was found for 368 (37%), a similar proportion to that described in this study. The Finnish study did not, however, test for streptococcal infection, and serological methods were used instead of culture for identification of adenovirus and enterovirus infections. Despite these differences the findings are remarkably similar and parvovirus B19 was also the most commonly identified infection. The relative proportion of rash-fever illnesses owing to each cause is likely to vary with the relative incidence of these infections. For example, this survey took place during 1997, a year with high activity for parvovirus B19,[20] but a relatively lower incidence period for scarlet fever.[21] In years where there is a lower incidence of parvovirus B19 infection, this is unlikely to account for such a high proportion of rash-fever illness. The remaining cases with rash and fever where no infectious cause was identified may have been a result of failure to detect infection with the tests and samples used in this study.

The study was conducted in the context of routine clinical care with voluntary participation by interested primary care physicians. We believe that our data provide reasonable insight into the performance of these enhanced laboratory tests in normal clinical practice, although the study was not population based.

This study reaffirms the guidance that all suspected measles and rubella should be investigated and that alternative diagnoses should be considered. Other infections are more likely to be confirmed than measles, particularly if conjunctivitis is not a feature. A saliva assay for parvovirus B19 is under development, but other infections can be excluded using routine investigations. GAS infection should be considered, particularly if sore throat is a feature and a throat swab for bacteriology is recommended. A positive finding may be an indication for antibiotic treatment, and knowledge of local circulation of GAS may alter the management of future cases.

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REFERENCES

1 **Cherry JD**. Contemporary infectious exanthems. *Clin Infect Dis* 1993;**16**:199–205.
2 **Bendig JWA**, Fleming DM. Epidemiological, virological, and clinical features of an epidemic of hand, foot, and mouth disease in England and Wales. *CDR Rev* 1996;**6**:R81–6.
3 **Gay NJ**, Hesketh LM, Cohen BJ, *et al*. Age specific antibody prevalence to parvovirus B19: how many women are infected in pregnancy? *CDR* 1994;**4**:R104–7.
4 **Miller E**. The new measles campaign. *BMJ* 1994;**309**:1102–3.
5 **Ramsay M**, Gay N, Miller E, *et al*. The epidemiology of measles in England and Wales: rationale for the 1994 national vaccination campaign. *CDR Rev* 1994;**4**:R141–6.
6 **Perry KR**, Brown DWG, Parry JV, *et al*. Detection of measles, mumps and rubella antibodies in saliva using antibody capture radioimmunoassay. *J Med Virol* 1993;**40**:235–40.
7 Laboratory diagnosis of measles infection and monitoring of measles immunisation: memorandum from a WHO meeting. *Bull World Health Organ* 1994;**72**:207–11.
8 **Ramsay M**, Brugha R, Brown D. Surveillance of measles in England and Wales: implications of a national saliva testing programme. *Bull World Health Organ* 1997;**75**:515–21.

9 **Public Health Laboratory Service**. *PHLS clinical microbiological standard operating procedure for the investigation of throat swabs.* B.SOP 9 issue 2. London: Technical Services, Public Health Laboratory Service Headquarters, 1996.
10 **Brown DWG**, Ramsay MEB, Richards AF, *et al.* Salivary diagnosis of measles: a study of notified cases in the United Kingdom, 1991–3. *BMJ* 1994;**308**:1015–17.
11 **Cohen BJ**. Detection of parvovirus B-19-specific IgM by antibody capture radioimmunoassay. *J Virol Methods* 1997;**66**:1–4.
12 **Ward KN**, Gray JJ, Fotheringham MW, *et al.* IgG antibodies to human herpesvirus-6 in young children: changes in avidity of antibody correlate with time after infection. *J Med Virol* 1993;**39**:131–8.
13 **Boom R**, Sol CJA, Salimans HMM, *et al.* Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* 1990;**28**:495–503.
14 **Yalcin S**, Karpuzoglu T, Suleymanlar G, *et al.* Human herpesvirus 6 and human herpesvirus 7 infections in renal transplant recipients and healthy adults in Turkey. *Arch Virol* 1994;**136**:183–90.
15 **Ramsay ME**, Brugha R, Cohen BJ, *et al.* Salivary diagnosis of rubella: a study of notified cases in the United Kingdom, 1991–4. *Epidemiol Infect* 1998;**120**:315–19.
16 **Tait DR**, Ward KN, Brown DWG, *et al.* Measles and rubella misdiagnosed in infants as exanthem subitum (roseola infantum). *BMJ* 1996;**312**:101–2.
17 **Baum SG**, Mandell GL, Bennett JE, eds. *Principle and practice of infectious diseases*, 5th edn. New York: Churchill-Livingstone, 2000:132,1624–30.
18 **Fox JP**, Hall CE, Cooney MK. The Seattle virus watch: VII. Observations of adenovirus infections. *Am J Epidemiol* 1977;**105**:362–86.
19 **Davidkin I**, Valle M, Peltola H. Etiology of measles and rubella like illness in measles and mumps and rubella vaccinated children. *J Infect Dis* 1998;**178**:1567–70.
20 Increased incidence of parvovirus B19. *Commun Dis Rep CDR Wkly* 1997;**7**:229.
21 **Public Health Laboratory Service**. Statutory Notifications of Infectious Diseases in England and Wales. PHLS web site 1996; http://www.phls.co.uk/facts/NOIDS/Annuals/