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Salivary diagnosis of rubella: a study of notified cases in the United Kingdom, 1991–4

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SUMMARY

Rubella infections, notified by general practitioners on the basis of a clinical diagnosis, were investigated by testing blood and saliva samples for specific IgM. Overall 52 (29%) of 178 cases with appropriately timed blood specimens were confirmed as recent rubella by IgM serology. Only 2 (3%) of 74 cases in children under 5 years were confirmed compared to 50 (48%) of 104 cases in older children and adults. The confirmation rate was lower (6%) in those with documented vaccination history than in those without (42%). The *specificity* of saliva rubella IgM testing compared to testing corresponding blood samples was 99%. The overall *sensitivity* of saliva rubella IgM testing was 81%. This rose to 90% if results from inappropriately timed specimens and specimens taking more than 1 week to reach the laboratory were excluded. A corresponding saliva rubella IgG test was 98% sensitive and 100% specific. Of 126 rubella IgM negative cases, 25 (20%) were positive for parvovirus B19 IgM. This study confirmed that rubella surveillance based on clinical reports is not specific. It also demonstrated that saliva samples, if taken 7–42 days after onset of illness and transported rapidly to the laboratory, are a feasible alternative to blood samples for rubella surveillance.

INTRODUCTION

The elimination of congenital rubella by the year 2000 is dependent on the maintenance of high vaccination coverage in children, and the identification and vaccination of non-immune women of childbearing age [1, 2]. Rubella vaccine has been used in childhood since 1988 as part of the measles–mumps–rubella (MMR) programme. In 1991 15–20% of males aged 5–16 years were found to be antibody negative [2] and therefore, rubella was included in the recent measles–rubella (MR) campaign in England and Wales in November 1994 [3]. The MR campaign should have reduced the large pool of male susceptibles, aged 5–16 years, but has not yet resulted in a reduction in the number of confirmed cases because most cases now occur in older age groups, especially in men [4]. These men are an important potential source of infection for susceptible pregnant women. The full impact of the campaign upon the risk of congenital rubella will not become apparent for some years [5].

In England and Wales, clinical cases of rubella are notified to the Office of National Statistics (ONS). Accurate surveillance is necessary to identify reservoirs of infection (because the clinical diagnosis of rubella is unreliable); to identify susceptible groups; for monitoring immunization programmes and for informing future policy. Laboratory confirmation of notified cases is required because, as the incidence of infection reduces, an increasing proportion of clinically diagnosed cases will be due to other diseases, e.g. human parvovirus B19 infection [6]. It has recently been shown that salivary antibody testing provides a

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reliable non-invasive alterative to serum testing for measles surveillance [7, 8]. This paper reports a community-based study of notified cases of rubella in England and Wales which took place in 1991–4. The study was designed to assess the sensitivity and specificity of salivary rubella-specific IgM and IgG in comparison with blood testing.

METHODS

Subjects and samples

Blood and/or saliva samples were collected from cases notified as rubella between August 1991 and February 1994 in 18 collaborating health districts. Consultants in communicable disease control (CsCDC) were provided with kits containing equipment for the collection of saliva and finger/heel prick blood samples from suspected cases. On receipt of a rubella notification, the CCDC sent a kit to the reporting doctor requesting samples from the patient, preferably within 1–6 weeks of onset of illness. Saliva samples were taken by the doctor or practice nurse at a home or clinic visit, after obtaining informed consent, by wiping a specially designed sponge swab around the gum margin for about a minute [9]. At the same time, a few drops of blood from a heel or finger prick were collected on to a filter paper strip. Saliva and blood samples were posted with a simple request form to the Public Health Laboratory Service (PHLS) Enteric and Respiratory Virus Laboratory. Vaccination histories of patients were obtained from general practice records and validated against health authority records. If vaccination was not documented in general practioner records, patients were assumed to be unvaccinated.

Laboratory methods

Saliva was extracted from the sponge swabs in 1 ml of phosphate buffered saline, pH 7·2, containing 0·2% Tween 20 and 10% fetal calf serum, by vortexing and centrifugation. Extracted saliva was stored at −20 °C before testing. Blood was extracted from a 0·5 × 0·5 cm area of filter paper into 200 μl of phosphate buffered saline. Saliva and blood samples were tested undiluted, and at a dilution of 1:100 respectively, by antibody capture radioimmunoassays for rubella-specific IgM (MACRIA) and IgG (GACRIA). The assays are based on tissue culture grown virus and monoclonal antibody, as previously described [7]. Test results were calculated as the total bound reactivity (counts per minute) of each specimen, divided by the mean bound radioactivity of 4 negative serum controls, and expressed as test negative (T:N) ratios. Specimens were considered IgM positive if they gave a T:N ratio greater than 3·0 and IgG positive if they gave of T:N ratio greater than 2·0. Blood samples negative for rubella-specific IgM were tested for human parvovirus B19 IgM using MACRIA as previously described [10].

RESULTS

Of 180 blood samples collected from notified rubella cases, 54 were rubella IgM positive. The results from 2 children aged 13 and 14 months, who had been vaccinated after the onset of illness, 2–4 weeks before the specimens were obtained, were excluded because the IgM may have been due to either rubella infection or the rubella vaccine (Table 1). After excluding these 2 cases, 52 (29%) of 178 cases were rubella IgM positive by blood testing. A documented history of rubella vaccine, either measles, mumps, rubella (MMR) or single antigen rubella, prior to the illness, was obtained from 63 (35%) of 178 from whom blood was obtained. Overall, a significantly higher proportion of non-vaccinated (48/115, 42%) than vaccinated (4/63, 6%) cases was confirmed as rubella-IgM positive in the blood sample ($P < 0{\cdot}001$), and this was consistent across most age categories (Table 1). Most cases were aged 10–24 years, and the confirmation rate was highest in this age group. Of the 178 notified cases, 74 (42%) were in children under 5 years, but only 2 (3%) were confirmed. By contrast, of 104 (58%) notified cases in older children and adults, 50 (48%) were confirmed. Four confirmed cases occurred in vaccinated children, 2 aged 1–4 years and 2 aged 5–9 years. These children had been vaccinated between 7 months and 5 years previously.

Paired blood and saliva were available for 177 cases. Overall there was agreement on 166 (94%) of the 177 paired samples (Table 2). Compared to the blood test the *specificity* of the saliva rubella IgM test was 99%, with only one false positive saliva result. The *sensitivity* of the saliva rubella IgM was 81%. The predictive value of a positive test for saliva rubella IgM in this population was 98% and the predictive value of a negative test was 92%. The 11 discordant results included 4 cases where the saliva specimens were taken outside of the appropriate illness-to-sampling interval (7–42 days) and an additional case

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Table 1. *Evaluation of notified rubella cases by age, vaccination status and confirmatory IgM blood test, England and Wales, 1991–4*

Age (years)	Not vaccinated			Documented vaccination			Total		
	No. tested	Confirmed		No. tested	Confirmed		No. tested	Confirmed	
		no.	(%)		No.	(%)		No.	(%)
< 1	20	0	(0)	1	0	(0)	21	0	(0)
1–4	16	0	(0)	37	2	(10)	53	2	(4)
5–9	17	6	(35)	18	2	(11)	35	8	(23)
10–14	21	12	(57)	3	0	(0)	24	12	(50)
15–24	26	24	(92)	2	0	(0)	28	24	(86)
⩾ 25	14	6	(43)	2	0	(0)	16	6	(37)
Unknown	1	0	(0)	0	0	(0)	1	0	(0)
Total	115	48	(42)	63	4	(6)	178*	52*	(29)

* Two cases with recent rubella immunizations were excluded.

Table 2. *Concordance of rubella IgM detection in blood and saliva from 177 cases of notified rubella, England and Wales, 1991–4*

Saliva IgM	Blood IgM				
	Positive		Negative		Total
	No.	(%)	No.	(%)	
Positive	43	(81)	1	(1)	44
Negative	10	(19)	123	(99)	133
Total	53*	(30)	124	(70)	177

* This includes one case where the specimens were taken 2–4 weeks after MMR vaccination.

where the sample was 10 days in transit to the laboratory. Exclusion of these cases raises the sensitivity to 90%. Paired blood and saliva samples from 176 cases were tested for rubella-specific IgG; 128/130 blood IgG positive cases were saliva rubella IgG positive (98% sensitivity) and all 46 blood rubella IgG negative cases were saliva IgG rubella negative (100% specificity).

Amongst 126 rubella IgM negative cases tested, 25 (20%) cases were positive for B19 IgM in the blood sample. Thus, overall, 14% (25/178) of notified cases examined by a blood test were B19 IgM positive. This accounted for 50% of reported rubella cases in those over 25 years. Cases with evidence of B19 infection were mainly older children and young adults: eight ⩾ 25 years, four 10–14 years, and nine 5–9 years. The temporal distribution of rubella notifications and of confirmed rubella and parvovirus B19 infections is shown in Fig. 1. During the first half of 1993 the proportion of confirmed rubella cases rose from 29 to

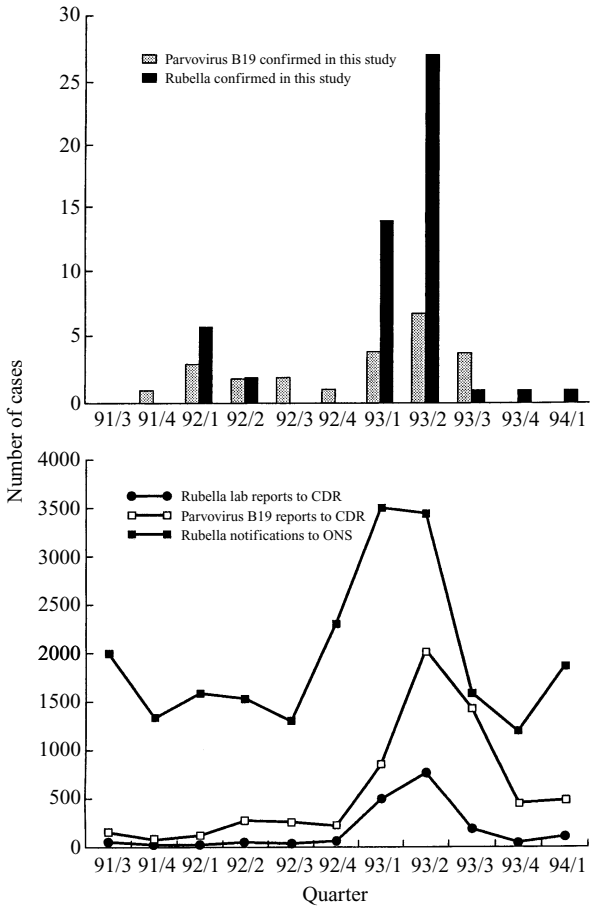

Fig. 1. Quarterly distribution of rubella and parvovirus B19 infections confirmed serologically during this study and laboratory reports to CDSC of rubella and parvovirus B19 and rubella notifications to ONS, England and Wales, 1991–4.

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67% and the proportion of cases diagnosed as parvovirus B19 rose from 14 to 18%, coinciding with a rise in the number of laboratory reports of rubella and B19 received by the PHLS Communicable Disease Surveillance Centre (CDSC) during this period (Fig. 1).

DISCUSSION

Since 1988, the national surveillance of rubella in England and Wales has included statutory notifications to the ONS. This is based on the clinical diagnosis made by reporting doctors and laboratory confirmation is not required. Clinical surveillance systems for rubella, however, are neither specific nor sensitive – due to failure to recognize cases clinically and under notification of suspected cases. In this study of rubella notifications from 18 health districts in 1991–4, we found that a relatively low proportion (52/178, 29%) was confirmed by rubella IgM test (Table 1). A further 14% were diagnosed as parvovirus B19 infections by demonstrating specific IgM in a blood sample. A surveillance system based on clinical reporting alone may therefore provide biased epidemiological data, especially in younger children: 42% of notified cases tested for rubella IgM were in children under 5 years, of whom only 3% were confirmed, compared with 58% reported in older children and adults, of whom 48% were confirmed as rubella. The low rate of confirmation in young children may be due to the unreliability of clinical diagnosis in this age group. Rubella is now rare in children and other paediatric infections presenting as rubella-like illness are relatively more common. In particular misdiagnosis of exanthum subitum (roseola, primary infection with human herpes virus, HHV6) may be a problem. A subset of 31 children, who were rubella IgM negative and under 2 years of age, were tested for HHV6 infection and 11 (35%) showed evidence of recent infection [12]. In addition, the possibility that young children may develop lower IgM responses, as demonstrated in parvovirus B19 infections presenting as erythema infectiosum [13], may make primary infection with rubella at this age more difficult to confirm serologically.

Alternative sources of data on rubella infections include serologically confirmed cases reported by laboratories to CDSC (Fig. 1). The lower acceptability which may be associated with the collection of blood specimens, particularly in children, means that these data are also biased. Obtaining saliva rather than blood specimens may be more acceptable to patients and clinicians and be logistically easier, resulting in a higher rate of compliance. The current study showed a high level of agreement between blood and salivary rubella IgM results, and the saliva test had a specificity of 99% (one false positive). The lower sensitivity of salivary testing was partly due to the false negative cases where the interval from onset of illness to sampling was outside the recommended period [7], and due to delays in the transport of specimens to the laboratory. A sensitivity of 90% can be obtained by ensuring that samples are taken 1–6 weeks after onset, and by sending saliva samples to the laboratory as soon as possible. Moreover, the adequacy of saliva samples for virus-specific antibody tests can be assessed by performing assays for total IgG and rejecting those with insufficient antibody for testing [14].

The sensitivity and specificity of rubella IgG testing of saliva specimens, following a mass rubella vaccination campaign in São Paulo, has recently been reported [15]. Saliva and blood rubella IgG test results in the present study showed a very high level of concordance, which confirms the reliability of saliva IgG testing and its potential value for epidemiological surveys and for targeting vaccination.

A single dose of rubella vaccine has been shown to be highly immunogenic [16, 17]. This study suggests vaccination is effective at preventing disease, with a 6% confirmation rate among vaccinated cases compared to 42% among unvaccinated cases. However, even with a 92% coverage in 2-year-olds since 1992 [18] and a 92% coverage in the recent MR campaign of 5–16-year-old school children [19] a substantial number of susceptible young adults (mainly male) still exists and a large outbreak of rubella took place in the UK during the first half of 1996 [20]. Rubella infection in these susceptibles may result in the exposure of susceptible pregnant women. A low threshold of clinical suspicion is therefore desirable in this age group and it is important to maintain reporting of suspected cases.

Future control measures for the prevention of congenital rubella are likely to include: the continued targeting of rubella vaccination for susceptible women of childbearing age; pre-conception counselling and the checking for a history of vaccination in school leavers; and the vaccination of susceptible immigrants. In addition, a second dose of rubella vaccine, given as MMR to all children before they start school at 4–5 years of age, was introduced into the UK

immunization schedule in October 1996. Such measures will require monitoring and cost effectiveness evaluations. As the incidence of rubella falls, the salivary testing of suspected cases is a simpler and more acceptable alternative to blood testing and will be increasingly important as an epidemiological tool for future rubella surveillance and control programmes.

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