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A school outbreak of parvovirus B19 infection investigated using salivary antibody assays

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SUMMARY

An outbreak of parvovirus B19 infection at a primary school was investigated using saliva samples. Antibody capture immunoassays for salivary B19 IgG and IgM were developed using a recombinant B19 antigen and monoclonal antibody to B19 virus. Evaluation of the salivary IgG assay using paired serum and saliva samples from 43 staff at St Thomas' Hospital showed that it had a sensitivity of 100% and a specificity of 95%. Evaluation of the salivary B19 IgM assay using 87 paired blood and saliva samples from a study of general practitioner rubella notifications showed it had a sensitivity of 60% and a specificity of 98%. Using the salivary assay the level of B19 IgG within 2 weeks of the start of the outbreak ranged from 5–33% in children and 29% in staff. By detecting salivary B19 IgM and/or B19 IgG seroconversions, attack rates of 8–50% in children in different classes and 47% in staff were observed. Household transmission was also studied and an attack rate of 45% was recorded in 11 susceptibles. After the outbreak, the level of B19 IgG in children with the highest attack rates was 60–70%, similar to that seen in adults in the UK. This study highlights the risk of B19 infection in an institutional setting and shows that saliva samples are a useful alternative to blood.

INTRODUCTION

Infection with human parvovirus B19 causes erythema infectiosum among children. In adults infection may result in acute polyarthritis and, during the first and second trimesters of pregnancy, hydrops fetalis and miscarriage. Furthermore, in patients with reduced red cell survival, B19 can cause transient aplastic crises and, in the immunocompromised, prolonged anaemia. At present laboratory diagnosis relies upon antibody detection in a serum sample [1]. However, as most infection occurs in childhood, serological tests which rely on non-invasive sampling may be more acceptable for the diagnosis of a normally trivial childhood infection. We have now developed, through advances in recombinant DNA technology in the production of B19 antigens coupled with the well-established antibody capture technique [1], a non-invasive sampling method based on salivary antibody assays capable of detecting recent and past infection with parvovirus B19. Here we describe how these assays were evaluated and employed to investigate an extensive outbreak of B19 infection in a primary school and the subsequent spread of infection within the home.

DESCRIPTION OF THE OUTBREAK

Notification of an outbreak of a rash illness among children in a primary school in south-west London was received by the local Consultant in Communicable Disease and Control (CCDC) in March 1994.

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Five children from two classes had recently developed a rash on the face and body which intermittently appeared and disappeared, during the preceding few days. Because of conflicting advice from General Practitioners regarding the cause of the rash, the requirement to keep affected children away from school, and the potential risk to pregnant contacts, the CCDC contacted the Department of Virology at Guy's and St Thomas' Hospital NHS Trust for assistance. Infection with parvovirus B19 was considered the likeliest cause because of the description of the rash and because it was an epidemic year for B19 [2]. The diagnosis was confirmed by detecting B19 specific IgM in a serum sample taken from one of the affected children aged 6 years who had developed a rash 3 days earlier, referred to as the index case. After discussion with the head teacher, school nurse and the CCDC it was agreed that saliva samples could be collected from all children and staff to determine the extent of spread within the school and then follow the spread of infection within their homes. Letters of consent were sent to all parents/guardians giving brief details about B19 infection including the possible adverse effects on the developing foetus. One of us (PR) was available to advise or allay anxieties resulting from this letter. Additionally an information leaflet designed by Communicable Disease and Surveillance centre (CDSC) was issued along with a copy of the parental letter to all GPs in the area outlining our intentions for the proposed investigation.

SCHOOL AND HOUSEHOLD STRUCTURE

The primary school contained a total of 242 pupils aged 3–11 years. Of these, 50 pupils aged 3–4 years, were in a separate nursery building. There was no direct connection between these buildings, and therefore, contact between pupils in the nursery and main school was minimal. The main school, comprising seven classes of 25–30 pupils each, had adjoining work areas separated by curtains. There were communal play and eating areas where all children in the main school were allowed to mix freely. A plan of the main school is shown in Figure. 1. The nursery comprised two classes of 25 pupils. The sex ratio in the main school and nursery was approximately 1:1. The entire school was staffed by 25 adults whose age ranged from 25–49 years with a M:F ratio of 1:7. The children's homes were a mixture of council-owned houses and flats and Victorian terraced accommodation.

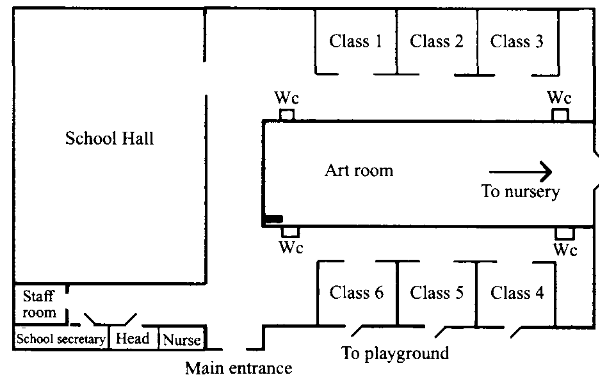

Fig. 1. Plan view of the main school.

MATERIALS AND METHODS

Sample collection

Saliva was collected with a specially designed foam swab by wiping the swab along the gingival margin as described previously [3]. Fluid collected by this method, although referred to as saliva, is actually a transudate of plasma proteins and therefore reflects antibody responses in blood [4]. One of us (PR) visited the school on two occasions and supervised the collection of samples by the children.

School children. Wherever possible saliva was collected by each subject on each occasion. The first batch of samples was collected 15 days after the onset of rash in the index case. The second was collected 39 days later to enable recent B19 infection to be determined by the presence of B19 IgM and/or B19 IgG seroconversion.

Household sampling. When the results of the first samples from the school children were known the parents of recent cases were contacted in order that saliva samples might be collected from other family members. A total of 20 adults (8 male, 12 female) and 9 additional children, age range 4 months–13 years, not attending the school because they were either too young or too old, were sampled from 11 households. Samples were obtained about 7 weeks (range 3–9 weeks) after the index case was diagnosed.

Samples used to evaluate the salivary B19 IgG and IgM assays. To validate the salivary B19 IgG assay, paired saliva and blood samples were collected from 43 healthy staff selected at random who were attending the Occupational Health department of St Thomas' Hospital for other reasons. This enabled the sensitivity and specificity of the salivary B19 IgG assay to be determined. To confirm that one saliva sample giving discrepant results had been collected adequately the quantity of total IgG was assayed by ELISA [5].

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To validate the salivary B19 IgM assay paired saliva and blood samples collected during a study of General Practitioner notifications of rubella in children during 1992–3 were made available [6]. Blood samples were obtained by heel or finger prick, collected onto filter paper and eluted with phosphate buffered saline. Eighty-seven saliva/blood pairs negative for rubella IgM were tested, enabling the sensitivity and specificity of the salivary B19 IgM assay to be determined.

B19 IgG and IgM antibody assays

Upon receipt in the laboratory saliva was extracted from the sponge swabs into 1 ml of phosphate buffered saline, pH 7·2, containing 0·05% Tween 20 and 10% foetal calf serum, by vortexing and centrifugation. Extracted saliva was stored at −20 °C before testing.

Radioimmunoassays for B19 IgG and IgM were performed by an antibody capture technique [1] with the following modifications when testing saliva samples: polystyrene beads coated with anti-u (Dako Lot no. 030) diluted 1/10000 or anti-γ (Dako Lot no. 027) diluted 1/2000; extracted saliva samples were added without further dilution and incubated for 2 h at 37 °C; baculovirus expressed B19 VP1 and VP2 capsids (Lot no. 911227, kindly provided by Dr Satchiko Kajigaya, NIH Bethesda) were used as antigen at a dilution of 10^{-4} and monoclonal antibody 521-5D (kindly provided by Dr Larry Anderson, CDC Atlanta) was used at a dilution of 10^{-6}. Controls comprising positive sera diluted to contain from 100–1 arbitrary RIA units of B19 IgM or IgG and a negative serum control were tested with each assay to ensure it had performed adequately. The radioactivity bound by the negative control serum was used to derive a test to negative (T/N) ratio for each saliva sample. A T/N ratio of > 4·0 for IgG and IgM was taken as evidence of the presence of specific antibody.

For the validation of the salivary B19 IgG assay corresponding serum samples were tested by a commercial ELISA (Dako Diagnostics Ltd) according to the manufacturer's protocol.

Case definitions

(i) Recent infection was defined as the presence of B19 IgM in either sample, with or without the presence of B19 IgG, or B19 IgG seroconversion.

(ii) Prior immunity was defined as the presence of B19 IgG in the absence of B19 IgM.

(iii) Susceptibility was defined as the absence of B19 IgG and B19 IgM.

Sickness rates and histories of rash illnesses were determined from class registers.

RESULTS

Evaluation of the B19 salivary IgG and IgM assays

IgG assay. The distribution of counts given by the 187 saliva samples collected during the first school visit suggested that a T/N value of 4·0 provided a suitable cut-off value for distinguishing between positive and negative samples (Fig. 2). Indeed when a T/N ratio of 4·0 was used the distribution of salivary B19 IgG revealed two distinct populations (Fig. 2). As a result of paired saliva and serum samples obtained from 43 members of staff at St Thomas', it was found that the assay was able to detect accurately evidence of past B19 infection. When compared with EIA on serum the sensitivity and specificity of the salivary B19 IgG antibody test were 100% and 95·5% respectively (Table 1). The saliva from only one subject gave a discrepant result – serum IgG −ve/saliva IgG +ve. The saliva from this subject contained 8·8 mg/l of total IgG, indicating that sufficient total immunoglobulin had been collected.

IgM assay. From the distribution of counts given by the 187 saliva samples collected during the first school visit it was difficult to discern a suitable cut-off value as the results did not form two distinct populations (Fig. 2). A T/N of 4·0 was, however, chosen as in the B19 IgG assay. As a result of testing 87 paired saliva and blood samples from patients notified as cases of rubella by General Practitioners, B19 infection was confirmed in 20 children by the presence of B19 specific IgM in blood eluted from filter papers. When compared with RIA on blood, by using the T/N ratio of 4·0 as a cut-off value, the sensitivity and specificity of the salivary B19 IgM assay was 60% and 98·4% respectively (Table 2).

Immunity, acute cases and illness in pupils

Salivary samples were obtained from 165 (68%) pupils on the first visit to the school and follow-up samples were obtained from 141 (85%) of these children. An additional 15 children had a single sample taken on this follow-up visit. Only one parent refused permission for sampling to be performed on his child.

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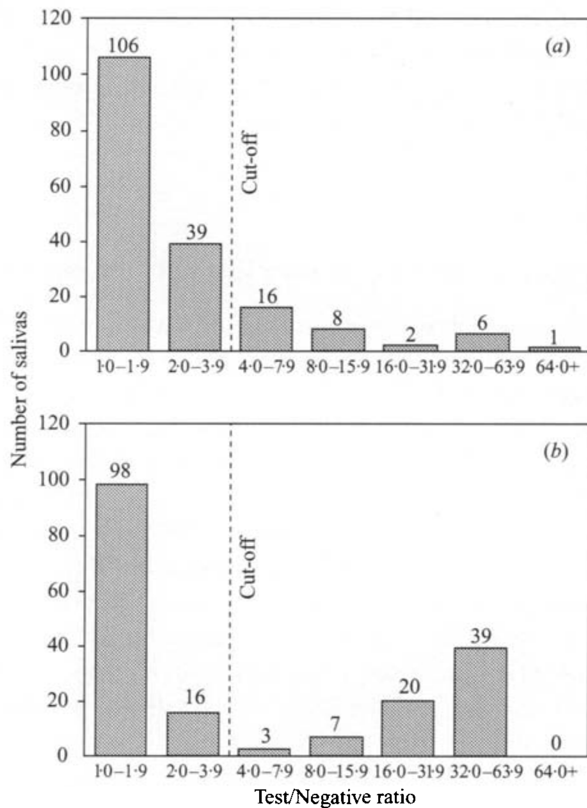

Fig. 2. Distribution of B19 IgG and IgM T/N values. T/N = Test/Negative ratio. Results: (*a*) IgM; (*b*) IgG.

Table 1. *Detection of B19 IgG in serum and saliva samples from healthy hospital staff (serum/saliva pairs* n = 43)

		Saliva	
		RIA positive	RIA negative
Serum	EIA positive	21	0
	EIA negative	1	21

Sensitivity of saliva assay = 100% (95% CI 83·9–100).
Specificity of saliva assay = 95·5% (95% CI 77·2–99·9).

The level of immunity to B19, as defined by the presence of B19 IgG in the absence of B19 IgM, and the number of acute cases, with attack rates by class and age group is shown in Table 3. Overall, 22% of pupils in the main school aged 4–11 years were defined as immune compared to only 6% in the nursery. However, among children aged 6–7 years in class 2, there was only one immune pupil (5%) when the first sample was collected.

There was a total of 35 acute B19 infections in pupils with 18 cases in boys. Recent B19 infections (as

Table 2. *Detection of B19 IgM by MACRIA in serum and saliva samples collected during a rubella notification study (blood/saliva pairs* n = 87)

		Blood		
		Positive	Equivocal	Negative
Saliva	Positive	12	0	1
	Negative	8	4	62

Saliva B19 IgM cut-off T/N ratio = 4·0.
Sensitivity of the salivary assay compared to blood = 60% (95% CI 36·1–80·9).
Specificity of the salivary assay compared to blood = 98·4% (95% CI 91·5–100).

defined previously) were seen in both the nursery and main school from 8 March until 11 April 1994 (Fig. 3). However, 32/35 (91%) acute cases occurred in the main school in pupils aged 6–11 years. Attack rates of 50% were seen in this age group except those aged 9–10 years in class 5, where an attack rate of only 13% was noted. This is illustrated in Figure 4, which also shows that at the end of the outbreak, the level of B19 IgG among children in the main school had reached that found in adults.

Of 35 acute infections, 27 (77%) were diagnosed by the presence of virus-specific IgM in the first saliva sample with 24 having both virus-specific IgM and IgG; of the three children who had only IgM detected, two later developed virus-specific IgG in a follow-up sample. Seven of the remaining eight acute infections (23%) were diagnosed as a result of seroconversion; five with IgM and IgG detected together in the second saliva and two seroconverting with only IgG. The remaining case had only a single sample collected which contained B19 IgM.

A total of 16 children reported a rash during the study period from the nursery, the reception class and classes 1–3. Of these, seven were confirmed as acute B19 infection and of the nine others, six had a follow-up sample collected revealing that they were still IgG seronegative. All five of the 30 children in class 2 who reported a rash were confirmed as recent B19 infection, compared to only one child in class 1, where six of 30 children reported a rash.

Of the 15 children sampled on the second occasion who were not at school when the first sample was collected, only one was found to have been recently infected with B19 and of the others, eight remained seronegative. Of the six children found to be B19 IgG positive but B19 IgM negative only one reported a

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Table 3. *B19 immunity* and attack rates in pupils and staff*

Class	Age (years)	Number immune/ number tested	Immune (%)	Number acute infections/number susceptible	Attack rate (%)
Nursery	3–4	2/35	6	3/32	9
Reception	4–5	4/16	25	1/12	8
One	5–6	5/26	19	2/21	8
Two	6–7	1/20	5	9/19	47
Three	7–8	8/24	33	8/16	50
Four	8–9	3/15	20	6/12	50
Five	9–10	4/19	21	2/15	13
Six	10–11	4/12	33	4/8	50
Staff		6/21	29	7/15	47

* As defined by the presence of B19 IgG in the absence of B19 IgM.

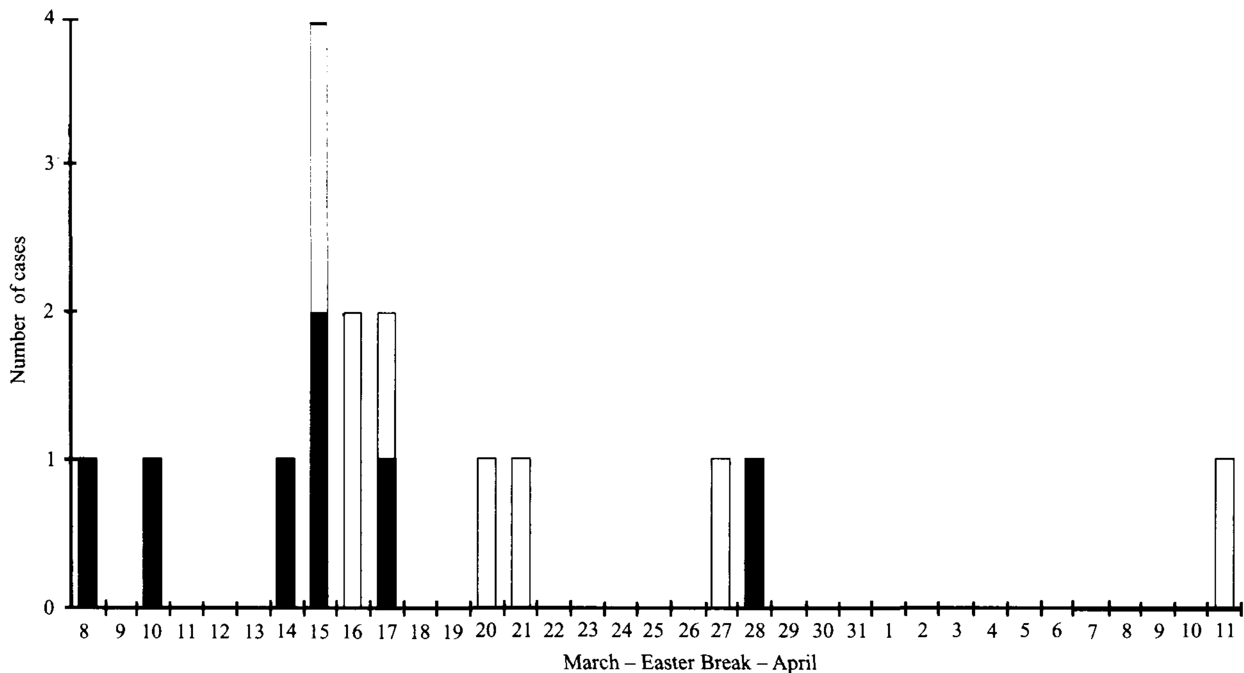

Fig. 3. Cases of B19 infection (■) and rash among children during the outbreak; □, rash not due to B19.

recent illness. Thus it is unlikely that the reason these children were not at school for the first sample was because they were at home with symptoms of B19 infection.

The follow-up samples taken approximately 5 weeks after the first samples allowed the persistence of B19 IgG and IgM to be estimated. Of the children with prior immunity who provided two samples, 46/49 (94%) had detectable B19 IgG detected in the second sample. This suggested that saliva collection was adequate as the B19 IgG assay had good repeatability. In addition, of the 27 acute cases with B19 IgM detected in the first sample, only one was found not to contain B19 IgG in the follow-up specimen.

Of those diagnosed as acute B19 infection 60% still had detectable B19 IgM by the time of the second sample 5 weeks later. No relationship between dates of rash onset and the persistence or disappearance of B19 IgM at 5 weeks could be ascertained by examining histories of rash from the class registers.

Immunity, acute cases and illness in school staff

Six (29%) of 21 staff were immune at the time of the first sample. Of 15 susceptible staff seven became infected during the study period, an attack rate of 47%. An illness presenting as rash and/or arthralgia reported by five members of staff was diagnosed as acute B19 by salivary antibody assays. Two additional

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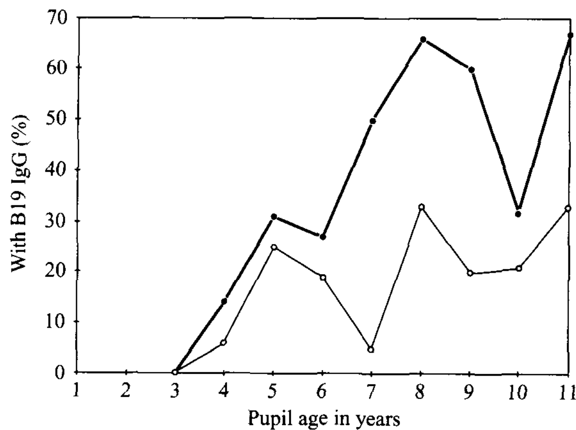

Fig. 4. Presence of B19 IgG among pupils in the first* and second† saliva samples. ○, first saliva; ●, second saliva. * The first saliva samples were collected 15 days after the rash in the index case. † The second saliva samples were collected 54 days after the rash in the index case.

cases diagnosed serologically remained asymptomatic. From a total of seven acute cases (five teachers, one meal supervisor and one clerical officer) six were diagnosed on the first sample by the presence of B19 IgM and B19 IgG and one as a result of B19 IgG and IgM seroconversion. No illness was reported among any other members of staff and none of the staff was pregnant. All cases occurred in women. Of the three male members of staff, two were immune when first tested and one remained susceptible at the end of the outbreak.

The duration of the IgM response was assessed. This showed that 33% of adults remained B19 IgM positive in the second saliva sample after an interval of 5 weeks.

Household infection

Once pupils had been identified as having had recent B19, spread of infection within their homes was examined. Twenty adults and nine children provided adequate saliva samples for B19 testing.

Nine (43%) were immune, six were susceptible and recent B19 infection was identified in five (F:M = 4:1) who were B19 IgG and IgM positive, giving an attack rate among susceptibles of five out of 11 (45%). Symptoms of rash and/or arthralgia were reported in four (80%; F:M 3:1) of the five adults with recent B19. Of four families with two affected children, all had symptomatic secondary cases in adults. This compares to only one adult case, who was a single parent in seven families with a single affected child at home.

No further cases could be confirmed among the nine children in these homes, possibly as a result of the late collection of samples and the short duration of the salivary IgM response. Indeed, the unexpected high level of immunity in these children, 7/9 (78%) were B19 IgG positive, is consistent with this possibility.

DISCUSSION

Our study has shown for the first time, the value of employing a salivary antibody assay to detect both past and recent infection with parvovirus B19. This assay allowed the determination of attack rates in a school outbreak without recourse to extensive blood sampling. This enabled a large number of young children to be recruited into the study. Moreover, because of its non-invasive nature, follow-up saliva samples could easily be collected thus permitting the diagnosis of an additional eight (23%) cases of B19 infection. This would not have been possible had blood samples been required, a requirement that may have hindered investigations of similar school outbreaks in the past [7, 8]. Additionally, the salivary antibody assay may facilitate the investigation of viral infections with longer incubation periods where repeated sampling is necessary as has been shown in a primary school outbreak of hepatitis A [9].

At the time of the first sampling the level of B19 IgG in the school children varied between classes ranging from 5 to 33%. This agrees well with an antibody prevalence study conducted in England and Wales using serum samples which found that children aged 0–4 and 5–9 years had levels of immunity to infection of 16 and 30% respectively [10]. In addition, only 29% of staff were found to be immune shortly after the outbreak had been notified. This is a low figure compared to an IgG prevalence of 50–60% in adults found in other surveys where serum samples have been used [1, 10]. Nevertheless, we believe the level of immunity detected among staff before the outbreak to be accurate as they experienced a similar attack rate to pupils and once the outbreak had subsided, immunity among the staff rose to 65%. Similarly, a low level of immunity (43%) was found in adult household contacts, though again, after the acquisition of acute B19 in this group, 67% were immune.

The overall attack rate of 50% amongst both pupils and school staff re-emphasises the risk that B19 infection poses to susceptible individuals and concurs with another study of a school outbreak where finger

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prick blood samples were used [11]. We found, as previously documented [12], that B19 infection also occurs in non-teaching staff (a meal supervisor and a clerical officer). Comparable attack rates of approximately 25–67% have also been documented in hospital outbreaks [13–15].

Although the differing attack rates in the nursery and main school may be explained by the two buildings being completely separate, it is difficult to explain why those children in class 5, adjacent to classes 4 and 6 with attack rates of 50%, had an attack rate of only 13% despite similar levels of pre-existing immunity in all three classes. However, another primary school outbreak study, conducted prior to the availability of serological assays for B19, found that two classes had clinical attack rates of less than 10%, despite rates of 25–35% in most classes [16].

Although most B19 infections were diagnosed on the first sample, a further eight (23%) were detected by testing follow-up samples. A few days after the first samples were collected the children went home for a 2-week break. It is possible that this may have terminated the outbreak as documented in a previous study [8]. Alternatively, the majority of infection may already have taken place prior to collecting the first sample.

Salivary antibody assays have been successfully used previously in the investigation of outbreaks of hepatitis A, sporadic measles and in seroprevalence studies of infection with hepatitis B and HIV [17–20]. It is important, however, that before a switch from blood to saliva for B19 infection testing is made, the limitations of the salivary assays are considered.

Positive and negative results in the salivary B19 IgG assay in children segregated into two distinct populations (Fig. 2) which enabled a cut-off value ($T/N = 4{\cdot}0$) to be clearly determined. The assay had a sensitivity of 100% and a specificity of 97% when compared to an ELISA test on corresponding serum samples. The performance of the salivary B19 IgM assay, however, showed that it may have lacked sensitivity. In the evaluation with paired saliva and blood specimens obtained from a rubella notification study, the salivary B19 IgM assay had a sensitivity of only 60% (though specificity of 98% was acceptable). This may be a low estimate due to the timing of specimen collection in the rubella notification study where samples were obtained on average 22 days after onset of rash [6]. In our study they were collected on average 11 days after onset. As expected, during an evolving outbreak, the distribution of B19 IgM results did not fall into two distinct populations (Fig. 2). This made it difficult to set a cut-off point for the B19 IgM assay. The cut-off value ($T/N = 4{\cdot}0$) may have been too high as demonstrated by the index case who was B19 IgM negative in saliva ($T/N = 3{\cdot}6$) only 13 days after a blood sample had been B19 IgM positive. This is consistent with previous findings that children with erythema infectiosum have low levels of serum B19 IgM [21]. A lower cut-off value ($T/N = 2{\cdot}5$) was considered but rejected. This was because it identified an additional 15 acute 'cases' of B19 of which 11 had B19 IgM only in the first sample with no evidence of B19 IgG in the same or follow-up samples. This led us to believe that the majority of these 'cases' were false positive and that we had set the cut-off for the B19 IgM assay appropriately at $T/N = 4{\cdot}0$. Therefore, despite the limitations of the IgM assay which need to be addressed in future developmental work, we believe salivary tests to be useful in the diagnosis of both recent and past B19 infection and possibly merit replacement of serum assays for investigating school or hospital outbreaks for several reasons.

First, in healthy adults as the results of a saliva/serum comparison showed it was possible to reliably detect past infection with B19.

Secondly, although we did not measure total immunoglobulin levels in the children's saliva samples, we are confident that the majority of samples were collected satisfactorily as there was a 94% repeatability for B19 IgG being detected in the first and second samples.

Thirdly, the attack rate of 50% and the age of children most commonly infected as determined in this study by salivary antibody assays compares very well with other outbreak studies in institutions such as schools and hospitals where serum samples were used [11, 13, 14]. Our results are therefore consistent with the known epidemiology of B19 infection and, as Figure 4 shows, by the time the outbreak had finished, the level of immunity among pupils, excepting class 5 (9–10 years), was very similar to that in the adult population [1, 10].

Fourthly, only seven of the 16 cases of rash reported in children could be identified as recent B19 infection leaving nine undiagnosed. Six of these were proven not to be due to infection with B19 as they remained seronegative for both IgG and IgM. This is reminiscent of a previous study using saliva to diagnose measles where only a minority of the rash illness reported as measles could be confirmed by the

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laboratory [3], illustrating the inaccuracy of clinical diagnosis of childhood exanthems. Assays for B19 infection should, therefore, be included in the surveillance of measles and rubella based on salivary testing.

As with other studies of B19 outbreaks [11], ours has shown that a large proportion of infections in children are asymptomatic or mild, but in adults there is a much higher clinical attack rate. Because the natural history of B19 infection is such that virus excretion has ceased by the time the rash appears, infection control is difficult. In spite of this it is important to establish the aetiology as appropriate advice may then be given to the school staff and parents, in particular pregnant women. The results of our study suggest that it is now feasible to use salivary tests for this purpose.

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