Infection with wild-type mumps virus in army recruits temporally associated with MMR vaccine

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SUMMARY

Four cases of mumps were reported among 180 army recruits who had received MMR vaccine 16 days earlier. Mumps serology, salivary mumps IgM and PCR tests for the SH gene were performed on the 4 cases and on 5 control recruits who remained well. PCR products were sequenced and the sequences compared to those of wild type and vaccine strains of mumps. Further salivary mumps IgM tests were performed on the remaining 171 recruits. Mumps infection was confirmed in the 4 cases but not in the 5 controls. The controls had serological evidence of prior immunity. The SH gene sequence found in the 4 cases was wild type. Saliva tests identified 2 additional recruits with mumps IgM, one of whom had presented with suspected mumps 2 days before the MMR vaccine was given. Thus 6 (5 symptomatic and 1 asymptomatic) cases of mumps in army recruits recently receiving MMR vaccine were not due to the vaccine but to coincidental infection with wild-type mumps virus. The probable index case was revealed by salivary mumps IgM tests. This study highlights the importance of appropriate investigation of illness associated with MMR vaccination.

INTRODUCTION

It is important to monitor adverse events associated with measles, mumps and rubella (MMR) vaccination. For example, reports of mumps meningitis following MMR vaccination lead to a change in the strain of mumps virus used in the vaccine from Urabe to Jeryl Lynn [1]. Parotitis has been documented in less than 1% of children receiving MMR vaccine [2] but no rates have been reported in older age groups. Rash and fever were also reported (in 6–17% of children [2]) but in many cases this may have been due to intercurrent primary infection with human herpes virus-6 (roseola infantum) which was responsible for 39% of measles and rubella notifications in a study of infants under 2 years [3]. We report an outbreak of mumps in 4 (2·2%) of 180 army recruits receiving MMR vaccine. Since the clinical attack rate was higher than previously reported after MMR vaccine and outbreaks of common viral infections are well recognized in newly assembled military recruits [4], it was decided to investigate the source of the outbreak. For this study we used the recently introduced laboratory methods of salivary antibody testing and genotyping which have also been used to enhance measles diagnosis and surveillance [5, 6]. Mumps RNA was directly detected in clinical specimens by PCR using primers for the small hydrophobic (SH) gene. The SH gene is highly variable and has been used to distinguish vaccine and wild-type viruses and to establish the phylogenetic relationship between mumps virus strains [6–9].

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PATIENTS AND METHODS

The outbreak

Gurkhas have been recruited from Nepal into the British army since 1815. One hundred and eighty Gurkhas are recruited once a year at an army camp in Pokhara, Nepal where they receive the same immunizations scheduled for all British army recruits which includes MMR vaccine (Pasteur Merieux MSD, MMR II). Six days after transferring to the UK in early February, 1998 and 16 days after receiving MMR, 4 Gurkha recruits presented with mumps; all 4 had parotitis and 2, in addition, had orchitis. There were no cases of meningitis or abdominal pain suggesting pancreatitis. Serum amylase tests were not done.

Specimens

Acute and convalescent serum, saliva, throat swab and urine samples were collected 6 and 27 days after onset of symptoms from the 4 cases and, for controls, corresponding specimens were collected from 5 recruits who had received MMR vaccine at the same time but had remained well. To study the evolution of the mumps IgM response, additional blood and salivas were collected from the confirmed cases at approximately monthly intervals for 4 months. To identify any additional recent mumps infections which may have been asymptomatic, salivas were collected from the remaining 171 recruits 39 days after presentation of the first 4 cases.

Mumps antibody assays

Mumps-specific IgM in serum and saliva was detected by IgM-capture radioimmunoassay (MACRIA) [11]. The level of mumps IgM was expressed in terms of T/N (test/negative) ratio with a T/N $\geqslant 3$ being considered positive. Mumps-specific IgG was detected in serum by ELISA (Behring Diagnostics UK Ltd, Milton Keynes) with results expressed in terms of optical density (OD) values; OD $\geqslant 0.200$ being positive.

Mumps PCR and sequencing (genotyping)

Mumps RNA was extracted from 100 μl of specimen using the silica–guanidinum thiocyanate method and reverse transcribed into cDNA with random primers.

Vero cell culture grown Taylor strain of mumps was used as positive control. Nested PCR amplification of a 639 bp fragment encompassing the entire SH gene was carried out using two sets primers, SH1 (5′-AGTAGTGTCGATGATCTCAT) and SH2R (5′-GCTCAAGCCTTGATCATTGA) for the first round PCR, and SH3 (5′-GTCGATGATCTCATCAGG-TAC) and SH4R (5′-AGCTCACCTAAAGTGACA-AT) for the nested PCR. Gel slices containing the specific DNA band were excised and DNA purified using a commercial kit (Geneclean, BI0 101 Inc., USA). The purified DNA fragments were sequenced using primers SH3 and SH4R with sequencing kit (Applied Biosystems). The nucleotide (nt) sequence of the SH gene (318 nt) was analysed and phylogenetic tree was drawn using the Megalign programme (DNASTAR package). The above procedures were performed essentially as previously described for measles virus [6, 12].

RESULTS

Recent infection with mumps virus was confirmed in 4 cases but not in 5 controls by conventional serology (Table 1). Mumps IgM was detected in both acute and convalescent serum from the cases and IgG was also present with OD values rising from acute (range 0·703–1·539, mean 1·126) to convalescent sera (range 1·007–1·922, mean 1·537). The antibody status of controls was consistent with prior immunity: mumps IgM was not detected and IgG was present with OD values falling slightly from acute (range 0·483–1·115, mean 0·846) to convalescent sera (range 0·474–0·892, mean 0·724).

Recent infection was also confirmed in the 4 cases by salivary mumps IgM which remained positive for 2 months after onset of symptoms. At 3 months after onset, salivary mumps IgM was negative in 3 cases and low positive (T/N 4·3) in one case. Corresponding sera were negative in 1 case and low positive (T/N 3·2, 4·6, 3·6) in 3 cases. Mumps IgM levels in acute salivas (T/N range 36·9–56·1, mean 45·8) were higher than in corresponding serum samples (T/N range 14·8–27·6, mean 20·1). Of the remaining 171 recruits, 2 were positive for salivary mumps IgM. One of them had a history of suspected mumps with an onset date 2 days before all the recruits were given MMR.

Mumps PCR was positive on acute saliva from all 4 cases (Fig. 1). Acute throat swabs were PCR positive in 2 subjects and in 2 others (who presented with orchitis) acute urine samples were PCR positive (Fig. 1).

Table 1. *Mumps IgM and IgG serology in cases and controls*

	Mumps IgM RIA T/N*		Mumps IgG ELISA OD†	
	Acute‡	Convalescent§	Acute	Convalescent
Case				
1	17·8	15·4	0·703	1·007
2	20·3	21·1	0·922	1·674
3	21·6	27·6	1·337	1·922
4	14·8	22·0	1·539	1·546
Control				
1	1·3	1·0	0·723	0·589
2	1·3	0·7	0·483	0·474
3	1·3	1·0	1·115	0·889
4	1·4	1·3	0·874	0·777
5	1·8	1·5	0·923	0·892

* T/N Test/negative ratio $> 3·0$ is positive.

† OD, corrected optical density, $OD_{450/620}$ nm $> 0·200$ is positive.

‡ Acute serum collected 6 days after onset of symptoms (22 days after MMR vaccination).

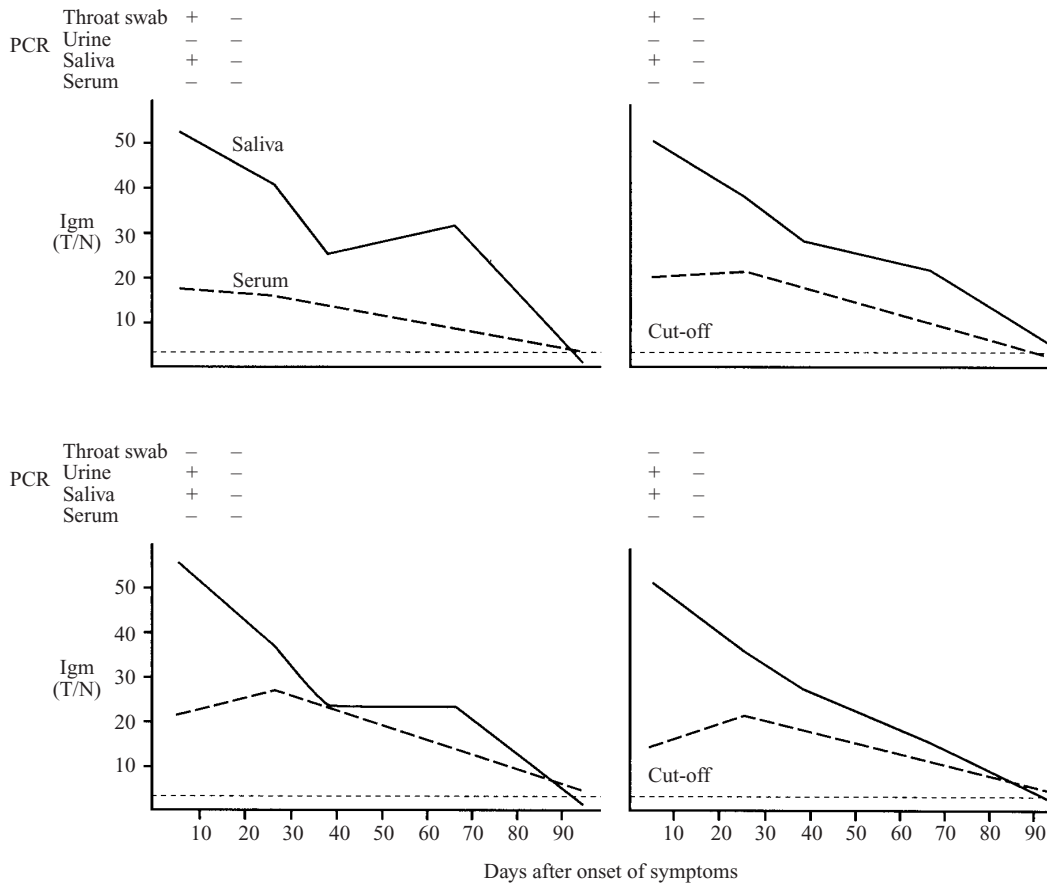§ Convalescent serum collected 27 days after onset of symptoms (43 days after MMR vaccination).

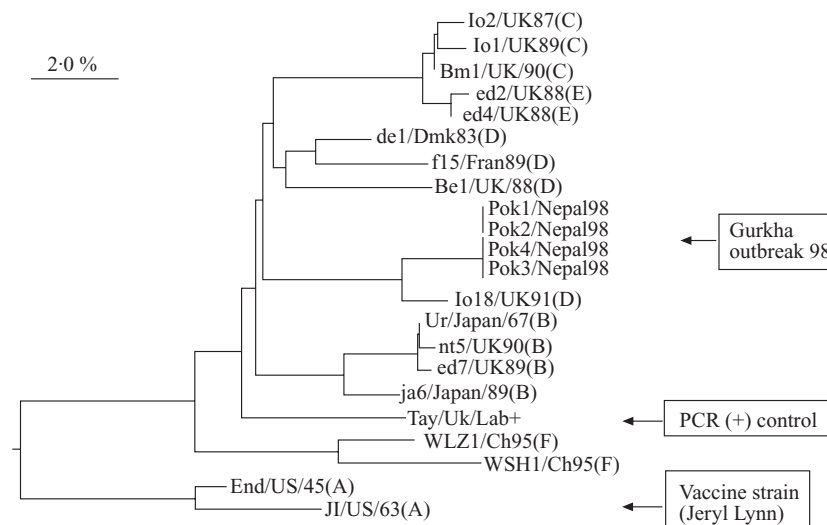**Fig. 1.** Mumps IgM and PCR results in four cases.

Fig. 2. Sequence relationships between genotypes (A–F) of mumps virus and the strain detected in the Gurkha recruits based on analysis of the SH gene (318) nt [13].

All sera and all of the convalescent samples were PCR negative, as were all samples from controls.

Identical sequences were detected in PCR product from saliva, throat swab and urine samples of the 4 cases which were distinguishable from sequences of the vaccine strain (Jeryl Lynn) and the laboratory strain used as PCR-positive control (Taylor, Fig. 2). Comparison with sequences of published mumps strains [13] showed that the strain identified in this outbreak was closely related to a wild-type genotype D strain identified in the UK in 1991 (lo18/UK91, Fig. 2).

DISCUSSION

The outbreak of mumps reported here was at first thought to be vaccine-associated since it occurred 16 days after MMR vaccination. Phylogenetic analysis of mumps virus from the outbreak showed it was related to other wild-type strains and not vaccine strains. It was most closely related to genotype D strains isolated in several European countries including the UK (Fig. 1). The infection could not, however, have been acquired in this country because symptoms were first reported only 6 days after the recruits had arrived in the UK and the incubation period of mumps is about 18 days. More extensive studies will be necessary to confirm the geographic range of mumps strains.

The clinical attack rate was higher than previously reported after MMR vaccine. Of 6 recruits with evidence of recent infection (i.e. mumps IgM positive) 5 had symptoms. One of these recruits had presented with mumps symptoms 2 days before the MMR

vaccine was given to all the recruits and may have been the index case for the outbreak. Vaccination 2 days after exposure to this case did not, however, prevent mumps disease in the 4 other symptomatic cases. The remaining 174 recruits remained well and probably had prior immunity since no IgM was detected either in response to MMR vaccine or exposure to wild-type virus. Prior immunity was confirmed in 5 control recruits from whom paired serum samples were collected (Table 1). The epidemiological findings highlight the phenomenon of outbreaks of common virus infections amongst newly assembled military recruits. Factors leading to such outbreaks include the gathering of young adults from diverse geographical and social origins and their accommodation in densely crowded quarters.

The laboratory diagnosis of recent infection with mumps virus was made by salivary IgM assay and by conventional serology. With these methods, however, it was not possible to distinguish a response to the mumps component of MMR vaccine from antibody developing as a result of infection with wild-type mumps virus. The source of infection was established only by PCR and sequencing. This showed that a wild type and not vaccine like strain was responsible for the outbreak and therefore demonstrated that the outbreak was not causally associated with MMR vaccination. The laboratory findings also illustrate how PCR and salivary IgM testing might also be useful in studies of the pathogenesis of mumps disease. Firstly, in the two infections complicated by orchitis, PCR was positive in urine, suggesting increased shedding of mumps virus from the genito-urinary

tract in these cases. Secondly, in the acute phase of illness, where corresponding saliva and serum samples were tested, mumps IgM reactivities were greater in saliva than in serum, suggesting local production of mumps-specific IgM.

Incompletely investigated reports of vaccine associated illness may have an adverse effect on public health because they reduce confidence in vaccination programmes. Recent reports of Crohn's disease [14] and autism [15], alleged to be associated with MMR vaccine, may have contributed to a reported fall in vaccine uptake [16]. As illustrated by the study reported here, laboratory tests are now available that allow adverse events reported after MMR vaccine to be completely investigated before being causally associated with the vaccination.

ACKNOWLEDGEMENTS

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