
Mumps outbreak in vaccinated children in Gipuzkoa (Basque Country), Spain

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(Accepted 11 July 2002)

SUMMARY

A mumps outbreak occurred in a group of vaccinated children aged 3–4 years in San Sebastián (Gipuzkoa, Basque Country, Spain) in 2000 during the same period as a revaccination campaign against measles–mumps–rubella (MMR) was performed. The clinical cases were confirmed by viral culture, detection of viral RNA and/or specific IgM. Eighty-eight percent of the children had been vaccinated with the Rubini strain and the remainder with the Jeryl–Lynn strain. The attack rate was 47·9% (35 cases in 73 school-attending children of this age). The outbreak was caused by an H genotype strain of mumps virus which was circulating at the same time as a D genotype strain that caused sporadic cases. By sequencing the small hydrophobic (SH) gene, the strains of the clinical cases were identified as wild-type mumps virus with heterologous genotypes in comparison to the vaccine strains used in our area.

INTRODUCTION

Mumps virus is the causal agent of epidemic parotitis and belongs to the genus *Rubulavirus* of the family *Paramyxoviridae*. Infection is usually asymptomatic or is associated with localized clinical expression in the parotid and salivary glands. Nevertheless, complications such as orchitis, meningitis and encephalitis occur frequently [1, 2]. Mumps vaccination was introduced in an increasing number of countries starting in the late 1960s, and subsequently there was a dramatic decrease in the incidence of this infection [2]. In some countries, such as Finland, introduction of a two-dose vaccination programme has led to elimination of the autochthonous infection [3]. Nevertheless, mumps outbreaks still occur in countries with a high level of vaccination coverage [2, 4, 5]. This study is an epidemiological and molecular analysis

of a mumps outbreak that occurred in San Sebastián (Gipuzkoa, Basque Country, Spain) in 2000 in a group of children that had been vaccinated. This outbreak occurred during a period in which there was increased incidence of mumps in Gipuzkoa.

METHODS

General data and vaccination status

San Sebastián is the capital of Gipuzkoa, a region with 695 855 inhabitants located in the Basque Autonomous Community (Northern Spain). In this region, the trivalent measles–mumps–rubella (MMR) vaccination was introduced in children of 15 months of age in 1981. Vaccination coverage has been >90% since 1987. Starting in 1991, a second dose was administered to 11-year-old children, with coverage of >87% as of 1994. In 2000, the age for administration of the second dose of the MMR vaccine was advanced

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to 4 years old, and a special vaccination was provided for children from 4 to 11 years old. In Gipuzkoa, as throughout Spain, mumps is a disease of required notification (EDO) that must be reported to the authorities. Following the last major epidemic that occurred in 1989 (439 cases per 100 000 inhabitants), the incidence of mumps in Gipuzkoa decreased gradually, with the exception of an epidemic peak in 1992 (65 cases per 100 000 inhabitants). There were only 1.9 and 2.2 cases per 100 000 inhabitants in 1998 and 1999, respectively [6].

Definition of probable and confirmed cases

A case of mumps was defined as a condition with unilateral or bilateral swelling of the parotid or other salivary glands, sensitive to touch and self-limited, with acute onset and duration of more than 2 days, without any other apparent causes. Confirmation of the case was based on laboratory diagnosis, including viral isolation, detection of mumps virus RNA by nested PCR (RT-nPCR) and/or detection of mumps virus-specific IgM. It was considered to be a probable case when there was an epidemiological link with a confirmed case.

Study of outbreak detected in 2000

In May 2000, a paediatrician reported two cases of mumps in children attending the same school class in the city of San Sebastián. Medical personnel from the Epidemiology Service performed a survey of all students in the class affected, and collected demographic (age, sex) and clinical (signs of mumps, date of onset, evolution) data on an individual basis. Verification that the MMR vaccination had been administered to each child was based on the vaccination card for each of the children as well as the vaccination register maintained by the Department of Health of the Basque Government, which state the date of vaccination and the type of vaccine used.

Clinical specimens for culture and/or detection of viral RNA

Between May and August 2000, the Microbiology Service received 55 clinical specimens for detection of mumps virus (18 saliva specimens, 20 throat swabs and 17 urine specimens) from 23 patients. Twelve patients were from the school where the reference case for the outbreak was reported and an additional

11 patients were unrelated to this case. The throat swabs were placed in viral transport medium (Hank's balanced salt solution containing gelatin and gentamicin), whereas the other specimens were sent in sterile containers without transport medium. The specimens were immediately cultured following arrival at the laboratory, or freezing at -70°C for 1–7 days for detection of the small hydrophobic (SH) gene of mumps virus.

Cell culture

Prior to culture, the specimens were mixed with an antibiotic mixture containing gentamicin, vancomycin and amphotericin B. Three hundred microlitres of the specimens were inoculated onto two shell vials with MDCK and LLC-MK2 cell monolayers, respectively, and processed according to the previously described methods with minor modifications [7]. The monolayers were stained by indirect immunofluorescence using a monoclonal antibody for mumps virus (Mumps-IFA kit, Chemicon International, USA).

RT-nested PCR

Viral RNA was obtained from the original specimen by using an extraction method with phenol-chloroform (TRIzol LS Reagent, Life Technologies, Gibco, USA) in accordance with the manufacturer's instructions. The transcription of RNA to cDNA was performed with M-MuLV reverse transcriptase (USB, USA). Subsequently, nested PCR was performed with the conditions indicated by Wu et al. [8]. In positive specimens, amplification of 413 nucleotides was visualized on agarose gel with ethidium bromide.

Sequencing (genotyping)

The PCR product of the positive samples was sequenced in an ABI-310 automated sequencer (Perkin-Elmer, Applied Biosystems, USA). The nucleotide sequences were analysed, and compared to one another and to other sequences deposited in the GenBank using the software available at the Web site of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>).

Serological testing

Commercial enzyme immunoassays (Enzygnost Anti-Parotitis-IgG and IgM, Dade-Behring, Germany) were

performed in accordance with the manufacturer's instructions in order to detect the presence of mumps virus-specific IgG and IgM. Prior to the detection of IgM, the serum specimens were treated with anti-human IgG (RF-Absorbens, Dade-Behring).

RESULTS

Incidence of mumps in Gipuzkoa in 2000

The incidence of mumps in Gipuzkoa increased from an annual rate of less than 5 cases per 100 000 inhabitants in 1996–9 to 8.5 cases per 100 000 inhabitants in 2000. Of the 58 cases reported in 2000, 36 were males and 22 females. Fifty-two of the patients were under 14 years old and 6 patients were over 20 years old. Thirty-five cases involved children from 3–4 years of age from the school class affected by the aforementioned outbreak. Another 6 cases occurred in subjects who were not from this class but were directly related to these children. Therefore, the outbreak accounted for 70.7% (41/58) of the cases reported to the EDO system in 2000. The other 17 patients were sporadic cases that represent an increase in comparison to the number of cases reported in the previous 2 years. Of the 58 cases reported in 2000, 52 of the patients had been vaccinated (92.9% of the total; 100% of the subjects under 14 years old).

Epidemic outbreak in the school class: incidence and vaccination history

Thirty-five of the 73 children in the school year in which the first 2 cases of mumps were detected developed mumps (attack rate: 47.9%). All 73 children had been properly vaccinated. They were youngsters from 3–4 years old (born in 1996), and the children affected included 21 males and 14 females. The index case occurred in May, 15 days after the patient had visited a relative in the Canary Islands, a child who was hospitalized due to lymphocytic meningitis. The outbreak continued for 3 months, and the final case occurred in July. In all cases, the illness was minor, and none of the children affected experienced complications.

The 73 children in the school class affected by the outbreak had received MMR vaccine at the age of 12 months. Twelve subjects had received a second dose of the vaccine during this year. Two of the

subjects who were revaccinated developed mumps. Mumps virus was isolated and sequenced in one of these patients (the one who had been revaccinated most recently, 14 days prior to the first symptoms) in order to determine the possible association with the vaccination virus. In the 61 children who received a single dose of the vaccine, the attack rate was 54.1%. Fifty-one (87.9%) of the 61 children had been vaccinated with the Rubini strain, 7 patients received the Jeryl–Lynn strain, and no data were available regarding the type of vaccine used for the other 3 children. The attack rate was 57 and 43% for the children vaccinated with the Rubini and Jeryl–Lynn strains, respectively.

The other 6 cases of mumps reported within the context of the outbreak who were not from the school class affected were three children aged 3, 5 and 7 years, from the same school but different classes, a 21-year-old youth who used the sports facilities at the school on a daily basis and 2 parents of the children affected in the outbreak (a 44-year-old man and a 41-year-old woman).

Microbiological diagnosis

In the 23 patients with specimens submitted for culture and/or RT–nPCR between May and August 2000, mumps virus was detected in 14 (60.9%) subjects: 11/12 (91.7%) of the cases were affected during the outbreak, and 3/11 (27.3%) were sporadic cases. An additional subject with a sporadic case of mumps was detected by serological testing (presence of IgM). Table 1 shows the results of the microbiological diagnosis for these 15 subjects.

Genetic characterization of the mumps viruses detected

Based on sequencing of the SH gene, 17 strains of the mumps virus from 11 subjects were characterized (Table 1). In six cases, the gene was analysed in two different specimens and the same sequence was identified. In nine cases, the strain was classified as H genotype. Epidemiologically, all of them were related to the outbreak described. The nucleotide sequences were identical to one another and identical to the sequence of the YLB/Swz/95 strain of the mumps virus (GenBank accession no. U35849). In two other sporadic cases unrelated to the outbreak that developed the disease in July and August, the

Table 1. Data from 15 patients with confirmed mumps in May–August 2000 in Gipuzkoa. Results obtained in the serological and mumps virus detection tests performed on saliva, throat swab and urine specimens with shell-vial and RT-nested PCR methods

Case no.	Age (years)	Sex	Date of onset of symptoms	Type of case	Geno-type*	Acute phase mumps antibody		Culture results			RT-PCR results		
						IgG	IgM	Saliva	Throat swab	Urine	Saliva	Throat swab	Urine
1	4	M	6 June	Outbreak	H	P	N	P	P	P	P	P	N
2	3	M	8 June	Outbreak	NP	P	N	P	P	P	NP	NP	NP
3	4	F	22 June	Outbreak	H	NP	NP	P	P	P	P	P	N
4	3	F	22 June	Outbreak	H	NP	NP	P	N	N	P	P	N
5	3	M	22 June	Outbreak	H	NP	NP	N	P	P	P	P	N
6	4	M	22 June	Outbreak	H	NP	NP	P	NP	NP	P	NP	NP
7	4	M	23 June	Outbreak	H	NP	NP	P	P	N	N	P	N
8	3	F	26 June	Outbreak	H	NP	NP	P	P	P	P	P	N
9	21	M	29 June	Outbreak	NP	P	P	N	P	P	N	N	N
10	3	F	30 June	Outbreak	H	NP	NP	P	NV	P	P	P	P
11	4	M	3 July	Outbreak	H	NP	NP	NP	N	NP	NP	P	NP
12	6	M	10 July	Sporadic	NP	P	P	N	N	N	N	N	N
13	6	F	17 July	Sporadic	D	P	P	P	P	N	P	P	N
14	5	M	3 Aug	Sporadic	D	P	N	P	P	N	P	P	N
15	11	F	8 Aug	Sporadic	NP	NP	NP	P	N	P	N	N	N

NP, not performed; P, positive; N, negative; NV, not valid (toxicity of culture cells); M, male; F, female.

* H genotype, strain equivalent to the YLB/Swz95 strain; D genotype, strain equivalent to the Po17s/Portugal96 strain.

nucleotide sequence of the SH gene was identified as D genotype, with a similarity of 98 and 100%, respectively, in comparison to the Po17s/Portugal/96 strain (GenBank accession no. Y08215).

DISCUSSION

The increase in the incidence of mumps in Gipuzkoa in 2000 represented a departure from the decreasing trend recorded throughout the 1990s. As a result of this trend, Gipuzkoa had nearly achieved the objective of eliminating mumps from this region according to the WHO definition for the European region of annual incidence of <1 case/100 000 inhabitants [2]. The increase occurred in conjunction with an increased incidence of mumps in Spain [9–11]. It is quite probable that the school outbreak described in this study began in the Canary Islands. The duration of the outbreak was short, and it hardly extended beyond the pre-school class in which it was first reported. This may have been related to the fact that it occurred at the end of the school year, during the period in which a special vaccination campaign was conducted in the region with the MMR vaccine for children from 4 to 11 years old. There are two aspects of this outbreak

which are especially noteworthy: the high attack rate in the class involved (nearly 50%), in spite of the fact that the population affected had been vaccinated, and the fact that the children affected were quite young, from 3–4 years old; therefore, they had been vaccinated fairly recently (approximately 2–3 years earlier). This suggests that the efficacy of the mumps vaccine received by these children was quite limited. Most of them had been vaccinated with the Rubini strain (87.9%) used partially in Gipuzkoa between 1995 and 1998. Outbreaks with similar epidemiological characteristics (in vaccinated populations, with higher incidence and attack rates in children under 5 years old) have been reported in Spain during the period 1997–2000. Moreover, these have been associated repeatedly with previous vaccination with the Rubini strain [10, 12, 13]. As observed in other outbreaks and epidemics in Switzerland [14, 15], Portugal [5] and Italy [16] these outbreaks suggest that vaccination with the Rubini strain of the mumps virus provides limited protection from disease, less than that associated with the Jeryl-Lynn or Urabe strains [2, 12–16]. In this study, no significant differences were observed between the attack rate of children vaccinated with the Rubini strain in comparison to the Jeryl-Lynn

strain. However, the group vaccinated with the Jeryl-Lynn strain was small ($n=7$).

The aetiology of the outbreak described was confirmed by the microbiological analysis of the cases with clinical diagnosis of epidemic parotitis. The shell-vial method was used for isolation of the mumps virus, as well as RT-nPCR for detection of viral RNA [7, 17]. The confirmation of mumps using non-invasive samples (saliva, urine, throat swab) is particularly useful since the clinical progression of the illness is usually benign, with few complications. In our study, in three cases where mumps virus was detected by PCR and/or cell culture, acute phase mumps IgM was negative and mumps IgG positive, a pattern consistent with secondary infection. In this context, it is often difficult to confirm mumps by traditional method, which involves performing a venepuncture in order to conduct serological testing.

The SH gene of the mumps virus has been used widely for molecular characterization since it has the most variable viral genome sequence and the mumps virus is not subject to genetic recombination processes [8]. At present there are a significant number of sequences of the SH gene available, which can be used to identify the new isolates by establishing the phylogenetic relationship with those that have already been identified. Based on analysis of the SH gene sequence, ten genotypes, referred to as A–I, have been established for mumps virus [8, 18–23]. The nine isolates detected in the school outbreak were similar to one another and to the YLB/Swz/95 strain. This strain, which was referred to as a new lineage by Ströhle et al. in 1996 [24], belongs to the H genotype [21]. Strains of this genotype have been isolated in recent years in the United Kingdom and Switzerland [21, 24]. The two isolates obtained from sporadic cases were similar to the Po17s/Portugal/96 strain associated with the D genotype [19]. The fact that at least two different viral strains were circulating in Gipuzkoa in 2000 confirms that, presently the simultaneous circulation of strains from different genotypes within a geographical area is not unusual [20, 25, 26]. Based on characterization of these strains, the possible causal relationship between the vaccination virus and the school outbreak of mumps was ruled out. This relationship had been considered due to the fact that the outbreak occurred when the revaccination campaign was performed.

The genotypes of the mumps virus strains circulating in Gipuzkoa (genotypes H and D) were different from those used in the vaccination (the Jeryl-Lynn

and Rubini strains are genotype A). The lack of similarity between circulating and vaccination genotypes has also been observed in outbreaks in different European countries in recent years [21, 24, 25, 27]. The efficacy of the vaccine in providing protection from infection by heterologous genotypes is presently being researched. It has been suggested that, since there are differences in the neutralizing epitopes of different genotypes, the immunity provided by a specific strain may be less effective against strains from other genotypes [15, 28]. This might also explain the cases of reinfection by mumps virus [28, 29] and the vaccination failures in subjects vaccinated with the current monotype vaccinations. The lack of conclusive information on this subject also contributes to the importance of the genetic characterization of the viruses that cause mumps in vaccinated populations.

In conclusion, the identification of the strains of the circulating mumps virus as wild-type strains, with different genotypes than those of the vaccination strains used in our area, was established by molecular analysis. Based on such analyses, the circulation of at least two different strains at the same time was established. One of these strains was responsible for the school outbreak in a fully vaccinated population. The results obtained indicate that molecular analysis of the mumps virus is a useful method for evaluation of viral circulation in a specific area.

ACKNOWLEDGEMENTS

We thank Professor Emilio Pérez-Trallero for his helpful advice and comments, and Mariano San Jose for his collaboration in finding cases and collecting information.

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