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Detection of RNA of Mumps Virus during an Outbreak in a Population with a High Level of Measles, Mumps, and Rubella Vaccine Coverage▽

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The duration of mumps virus RNA detection was studied during a mumps outbreak in a highly vaccinated university population. Seven of the eight reverse transcription-PCR-positive specimens were collected during the first 3 days of parotitis, suggesting that viral shedding is minimal after the first 3 days of symptoms.

Over 6,000 mumps cases were reported in the United States during 2006 (3), the largest outbreak in two decades. Many cases had received two doses of mumps virus-containing vaccine; the highest incidence was observed among persons aged 18 to 24 years.

One strategy to reduce mumps transmission during outbreaks is patient isolation. Isolation has been recommended for 4 or 9 days (1, 5, 11, 13), based on data showing viral shedding up to 9 days after symptom onset. Ideally, the duration of isolation should be based on duration of viral shedding; however, viral shedding has not been studied in a population with high two-dose measles-mumps-rubella (MMR) vaccine coverage. Here we describe the timing and duration of mumps virus RNA detection in a highly vaccinated population during the 2006 outbreak.

Students from a university in Kansas with suspected mumps (pain or swelling of the parotid gland or jaw area or testicular pain or swelling) from 2 May through 13 May 2006 were evaluated. Blood and buccal swabs were collected at the time of evaluation. Attempts were made to collect repeated buccal swabs. Students with onset of physician-documented parotitis from 18 April through 1 May were asked to provide buccal samples the second week after onset. Inclusion criteria included physician documentation of parotid enlargement or tenderness or of testicular swelling or tenderness, without likely alternate etiology, or student report of parotid enlargement for ≥2 days or testicular pain or swelling. Students self-reported symptoms and demographics; vaccination status was determined from university records. Samples for virus detection were collected by massaging the parotid gland for 30 seconds followed by swabbing the Stenson's duct with a cotton swab. Swabs were placed in viral transport medium, immediately refrigerated, packed on wet ice, and shipped to the Centers for Disease Control and Prevention (CDC).

Thirty-one students participated; 21 (68%) provided a blood sample and nine (29%) provided repeat swabs (Table 1). The mean age was 20 years (range, 18 to 26 years); 17 (55%) were female, and 27 (87%) were non-Hispanic white. Thirty (97%) had received two MMR vaccine doses; one had one dose. Students with two MMR vaccine doses received the second dose a median of 13.7 years (range, 1.6 to 16.1 years) before symptom onset. Most reported parotitis (Table 2).

To isolate mumps virus, the buccal swab samples were inoculated onto Vero/SLAM cells, which are routinely used for isolation of measles, mumps, and rubella viruses (10). Unfortunately, because of problems with shipping, some of the samples arrived at CDC at room temperature and mumps virus was not isolated from any of the swabs.

Conditions adversely affecting viral viability do not necessarily result in degradation of viral RNA. Previous studies have shown that reverse transcription-PCR (RT-PCR) is at least as sensitive as virus isolation for detecting mumps virus (2, 4, 9, 12, 13). Numerous samples were submitted to CDC by multiple state laboratories during the mumps outbreak of 2006. All of the samples that were positive for mumps isolation at CDC were also positive when tested by RT-PCR, while mumps virus was not isolated from PCR-negative samples from clinically compatible cases (CDC, unpublished data). Therefore, we attempted to detect mumps viral RNA in the samples from the Kansas study by using a real-time RT-PCR that can reliably detect as few as 10 copies of mumps virus SH gene RNA (2). For the mumps primers and probes, a threshold cycle (C_T) of <38 was considered positive, a C_T of ≥38 and ≤40 was considered equivocal, and a C_T of >40 was considered negative. As a control for RNA extraction and integrity, the samples were tested for the mRNA for RNase P, a widely expressed cellular gene. RNase P was detected in all samples. The median C_T value for the RNase P assay was 30.55 for all samples, and the median C_Ts for the positive (30.52) and negative samples (30.63) were nearly identical, indicating that the concen-

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TABLE 1. Timing of sample collection by days of parotitis symptoms and results of RT-PCR and IgM and IgG assays

Student group and case no.[e]	Sex[f]	RT-PCR result by day of sample collection[g]:													Result of assay[a]		Duration of parotitis[h] (no. of days)
		1	2	3	4	5	6	7	8	9	10	11	12	13	14 and later (day)	IgM	IgG	
Detection																		
1	M	+														Neg	Pos	5
2	M	+														Neg	Pos	8
3	F		+													Neg	Pos	7
4	M		+													NA	NA	5
5	F		+		−		−									Neg	Pos	5
6	M			+												Neg	Pos	12
7	F			+												Pos	Pos	6
8[b]	M														+	Neg	Pos	0
No detection																		
9	F	−														Pos	Pos	6
10	F	−														NA	NA	5
11	M	−														NA	NA	4
12	M	−														Neg	Pos	E
13	F	−		−												Neg	Pos	5
14	F	−		−												Neg	Pos	8
15	F		−													Neg	Pos	T
16	F		−													Neg	Pos	6
17	M		−													Neg	Pos	9
18	F		−		−		−			−		−				Neg	Pos	5
19	M			−												Neg	Pos	8
20	M			−		−										Neg	Neg	12
21	F			−			−		−							Neg	Pos	6
22	F				−		−		−							Neg	Pos	T
23	F					−										Neg	Pos	T
24[c]	M						−		−		−		−		− (14)	Pos	Pos	8
25	F										−					NA	NA	10
26	M											−			− (15)	NA	NA	8+[d]
27	F												−			NA	NA	4
28	F													−		NA	NA	3
29	M														− (15)	NA	NA	7
30	M														− (15)	NA	NA	11
31	F														− (22)	NA	NA	T

[a] Blood specimens for IgM and IgG were collected at the time of the first swab. Neg, negative; Pos, positive; NA, data not available (blood sample not collected).
[b] This student had symptoms of swelling and pain in the testicles only; days are counted after onset of testicular pain.
[c] This was the only student with only one dose of MMR vaccine.
[d] This student reported symptoms at 8 days but was not reached for follow-up after 8 days.
[e] Detection, students for whom viral RNA was detected by RT-PCR; no detection, students for whom viral RNA was not detected by RT-PCR.
[f] F, female; M, male.
[g] +, positive specimen; −, negative specimen. Symptom onset is day 1.
[h] T and E, parotid tenderness or enlargement, respectively, was reported by physician, but student did not report duration of parotitis.

TABLE 2. Symptoms reported by students at any time during the course of illness

Symptom	No. (%) of students reporting symptom	
	All participants (n = 29)[a]	Cases who were PCR positive (n = 8)
Parotitis	25 (86.2)	7 (87.5)[b]
Jaw pain	26 (89.7)	7 (87.5)[b]
Swelling below the jaw	25 (86.2)	7 (87.5)[b]
Sore throat	20 (69.0)	6 (75.0)
Headache	18 (62.1)	4 (50.0)
Ear pain	18 (62.1)	5 (62.5)
Fever	15 (51.7)	2 (25.0)
Neck pain	14 (48.3)	3 (37.5)
Cough	13 (44.8)	2 (25.0)
Testicular pain and swelling	2 (15.4)[c]	1 (20)[c]

[a] Symptoms were not available for 2 of the 31 participants.
[b] One student without any parotitis, jaw pain, or swelling below the jaw who was PCR positive had orchitis only, without other symptoms.
[c] Percentage of males for whom symptoms were available.

trations of intact RNA in all of the samples were equivalent. Therefore, differences in the ability to detect RNA from mumps virus in the samples were not due to variation in the amount of RNA in the sample, the presence of nonspecific inhibitors, or the shipment conditions. Mumps virus RNA was detected in samples from eight (25.8%) students; all had received two MMR vaccine doses (Table 1). Seven reported parotitis, and one gave a history of testicular pain and swelling only and had a testicular nodule on examination. All seven students with parotitis and positive RT-PCR results had the swab obtained within the first 3 days of symptoms (Table 1). Mumps virus RNA was also detected from the buccal swab of the student with a testicular nodule, swabbed 13 days after orchitis onset (the student was asymptomatic at the time of swabbing). For the positive samples the C_Ts ranged from approximately 31 to 37, which is equivalent to a range of approximately 1,000 to 10 copies of mumps virus RNA per sample (2).

TABLE 3. Number of first samples collected by time since onset of parotitis with mumps viral RNA detected (positive) or not detected (negative) for students with parotitis or parotid tenderness

Days[a]	No. (%) of initial samples		
	Positive	Negative	Total
1–3	7 (35)	13 (65)	20
4–7	0 (0)	3 (100)	3
8–14	0 (0)	4 (100)	4
15–22	0 (0)	4 (100)	4

[a] Symptom onset is day 1.

Mumps viral RNA was not detected in 23 (74.2%) students (Table 1); 22 (95.7%) of these students had received two MMR vaccine doses; one had one dose. All 23 had parotitis, and one also reported testicular pain and swelling.

Of the 20 individuals with parotid enlargement or tenderness from whom at least one swab was collected within the first 3 days of parotitis symptoms, seven (35%) had a positive RT-PCR result (Table 3). The probability of detecting mumps virus RNA from the first swab was greater when the swab was collected during the first 3 days of symptoms (7/20) than when the swab was collected on days 4 to 14 (0/7; $P = 0.049$, Fisher's exact test).

There was no association between RT-PCR results from the first swabs collected and the report of any specific symptom on that day. The strongest association was with parotitis on the day of first swab (6/11 with parotitis had a positive swab, whereas 1/9 without parotitis had a negative swab; Pearson chi-square; odds ratio, 9.6; 95% confidence interval, 0.9 to 105.2). There was no association between RT-PCR results (from any swabs or those during the first 3 days) and fever or headache, possible indicators of the severity of the illness. There was no association with duration of parotitis or illness.

If the real-time RT-PCR gave a positive result, the complete sequence of the SH gene (318 nucleotides) was obtained (7) and the genotype was compared with SH gene sequences from reference strains recommended by the World Health Organization (8). All sequences from this study represented genotype G of mumps virus and were identical to each other and to the sequences of other genotype G mumps viruses isolated in other U.S. states and Canada (13) during 2005 and 2006.

Immunoglobulin M (IgM) specific for mumps virus was detected with an IgM-capture enzyme immunoassay that was developed using a protocol similar to that previously reported for measles virus IgM capture assay (6). Most students with RT-PCR-positive results were IgM negative and IgG positive (Table 1).

Our results suggest that detection of mumps viral RNA from oral samples of patients with two doses of MMR vaccine is generally limited to the first 3 days of symptoms. Although mumps virus was not isolated from any of the samples, all of the samples contained detectable mRNA from RNase P, indicating that RT-PCR provided a reliable and sensitive surrogate for virus isolation. Viral shedding might last longer in unvaccinated individuals or those with only one MMR vaccine dose. Our results support a recommendation for less than 9 days of patient isolation after onset of mumps symptoms in persons with two MMR vaccine doses.

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