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Comparison of the Sensitivity of Laboratory Diagnostic Methods from a Well-Characterized Outbreak of Mumps in New York City in 2009

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A mumps outbreak in upstate New York in 2009 at a summer camp for Orthodox Jewish boys spread into Orthodox Jewish communities in the Northeast, including New York City. The availability of epidemiologic information, including vaccination records and parotitis onset dates, allowed an enhanced analysis of laboratory methods for mumps testing. Serum and buccal swab samples were collected from 296 confirmed cases with onsets from September through December 2009. All samples were tested using the Centers for Disease Control and Prevention (CDC) capture IgM enzyme immunoassay (EIA) and a real-time reverse transcription-PCR (rRT-PCR) that targets the short hydrophobic gene. A subset of the samples ($n = 205$) was used to evaluate 3 commercial mumps IgM assays and to assess the sensitivity of using an alternative target gene (nucleoprotein) in the rRT-PCR protocol. Among 115 cases of mumps with 2 documented doses of measles, mumps, and rubella (MMR) vaccine, the CDC capture IgM EIA detected IgM in 51% of serum samples compared to 9% to 24% using three commercial IgM assays. The rRT-PCR that targeted the nucleoprotein gene increased RNA detection by 14% compared to that obtained with the original protocol. The ability to detect IgM improved when serum was collected 3 days or more after symptom onset, whereas sensitivity of RNA detection by rRT-PCR declined when buccal swabs were collected later than 2 days after onset. Selection of testing methods and timing of sample collection are important factors in the ability to confirm infection among vaccinated persons. These results reinforce the need to use virus detection assays in addition to serologic tests.

Mumps is an acute infectious disease, usually spread by droplet infection, with an incubation period of 16 to 18 days. Infection can be asymptomatic in about 30% of those who become infected. Prodromal symptoms are nonspecific and include fever, headache, myalgia, and malaise. Swelling of the parotid gland(s) is the most common clinical presentation in symptomatic cases. Generally, the disease is mild, but complications such as orchitis, encephalitis, and deafness can occur (1). Following the introduction of the measles, mumps, and rubella vaccine (MMR), the reported incidence of mumps declined from >100 cases per 100,000 population on average before 1967 (prevaccine era) to 10 cases per 100,000 population in 1977 (2).

Despite the sharp reduction in cases of mumps in the late 1970s following the adoption of universal childhood vaccination with 1 dose of mumps-containing vaccine, there were reports of outbreaks among vaccinated populations in the United States, affecting mainly school-age children from 1986 to 1990. The outbreaks were limited in both size and duration, and laboratory testing was generally conducted on only a small proportion of the cases (2–5). Therefore, the ability of routine diagnostic testing to confirm mumps infection among vaccinated individuals was not recognized as a problem. With full implementation of the 2-dose MMR vaccination schedule, reported cases of mumps and outbreaks declined further, to <0.1 cases per 100,000 persons from 2001 to 2005 (6).

In 2006, over 6,500 mumps cases were reported, primarily among young adults in the Midwest, many of whom had received 2 doses of MMR (7). The intensified surveillance for cases produced a surge in requests for laboratory testing from suspected mumps cases, and detection of IgM proved to be an unreliable marker of infection in outbreaks affecting highly vaccinated populations (7–10). A mumps real-time reverse transcription-PCR (rRT-PCR) method was developed and validated to improve the ability to confirm mumps cases (11). Although rRT-PCR proved to be more sensitive than IgM detection, negative rRT-PCR results were obtained from a substantial proportion of specimens submitted during the mumps outbreaks in 2006 (7, 9, 10).

A large mumps outbreak (3,502 cases) occurred from 2009 to 2010 (12, 13) among residents of Orthodox Jewish communities in New York City, New York State, and New Jersey. A high proportion of these cases occurred among individuals with 2 documented doses of MMR. Fifty-two percent of the cases in the outbreak occurred in New York City. The availability of serologic and virologic testing from a selection of confirmed mumps cases in New York City provided an opportunity to analyze the performance of a variety of laboratory methods and the impact of timing of specimen collection as well as vaccination status on test results.

MATERIALS AND METHODS

Suspected mumps cases are reported to the New York City Department of Health and Mental Hygiene (DOHMH), by clinical reports from providers and mandated electronic laboratory reporting (14). Information on age, gender, symptoms, symptom onset date, vaccination status, and date of specimen collection was collected as part of the public health investi-

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gation. Case reports were investigated and classified based upon the 2007 Council of State and Territorial Epidemiologists (CSTE) surveillance case definition (12). Confirmed cases met the clinical case definition (unilateral or bilateral tender, self-limited swelling of the parotid or other salivary glands without other apparent cause, lasting at least 2 days) or had clinically compatible illness and were either laboratory confirmed (positive mumps IgM by any assay, positive mumps culture, or positive mumps rRT-PCR test) or epidemiologically linked to a confirmed case. Symptom onset date was recorded as the date of parotitis onset or, for those with clinically compatible illness (facial, jaw, or neck swelling or orchitis), the date of onset of those specific symptoms.

Between 15 September and 21 December 2009, there were 782 case reports confirmed by DOHMH. Confirmed mumps cases that had a serum sample sufficient to complete testing as well as an adequate specimen for virologic testing (buccal swab) were included in the study ($n = 296$). These specimens were collected by providers in the community, based on guidance provided by DOHMH in the form of a 2-page flyer, "Mumps Guide for Providers" (15). In the document, providers were instructed to perform a 30-s parotid massage prior to specimen collection. Instructions were included for proper sample handling and storage.

Mumps vaccination status was based on documented, valid MMR doses. Acceptable documentation included MMR doses recorded in the New York City Citywide Immunization Registry (14) or those documented by a medical provider. In order to be considered valid, doses had to be administered no earlier than 4 days before the first birthday and at least 28 days after a previous MMR dose. Individuals lacking MMR documentation from a medical provider and with no record in the registry were considered to have unknown MMR vaccination status.

Serum samples from all 296 study cases were tested at the New York City Public Health Laboratory for mumps IgM using an indirect enzyme immunoassay (EIA) (Serion enzyme-linked immunosorbent assay [ELISA] classic mumps IgM; QED Bioscience, Inc., San Diego, CA). The serum samples were tested at the Centers for Disease Control and Prevention (CDC) by using a mumps capture IgM EIA; this assay was utilized in previous investigations of mumps outbreaks (7–10) and was developed and validated at the CDC using a protocol similar to that previously reported for the measles capture IgM EIA (16). Mumps-specific IgG was measured using a commercial EIA (mumps IgG ELISA II; Wampole Laboratories, Princeton, NJ). Because infections caused by Epstein-Barr virus (EBV) can cause parotitis and produce nonspecific reactions in mumps IgM assays (1, 17) (CDC, unpublished data), the serum samples were tested for heterophile antibodies to EBV (Monospot Latex, Meridian Bioscience, Inc., Cincinnati, OH).

Serum samples with sufficient volume from the cases that were confirmed by virus isolation (Fig. 1) (205/209) were tested by two additional methods for IgM detection, a commercially available capture IgM EIA (Microimmune Mumps IgM, BluePoint Bioscience, Ijamsville, MD), and an indirect immunofluorescent antibody (IFA) assay, performed in accordance with the manufacturer's directions (MBL Bion, Des Plaines, IL). Serum dilutions (1:10) for the IFA assay were prepared in Gullsorb (Meridian Bioscience Inc., Cincinnati, OH) to remove interference from IgG.

The buccal swabs were tested for the presence of mumps RNA using the rRT-PCR assay developed in 2006 (11) that targeted the short hydrophobic (SH) gene (rRT-PCR-SH). A variation of the rRT-PCR was developed during this study, with primers and probes directed to the nucleoprotein (N) gene (rRT-PCR-N), using the same reaction conditions. The RNA was extracted directly from the buccal swabs and purified as previously described (11). The sequences of the primers and probes for the rRT-PCR-N were as follows: forward primer, 5′-GTA TGA CAG CGT ACG ACC AAC CT-3′; reverse primer, 5′-GCG ACC TTG CTG CTG GTA TT-3′; probe, 5′-CC GGG TCT GCT GAT CGG CGA T-3′, with the 6-carboxyfluorescein (FAM) dye incorporated at the 5′ terminus and a BHQ1 quencher molecule at the 3′ terminus. The complete protocol is available on the CDC website at http://www.cdc.gov/mumps/lab/qa-lab-test-infect.html#realtime-pcr.

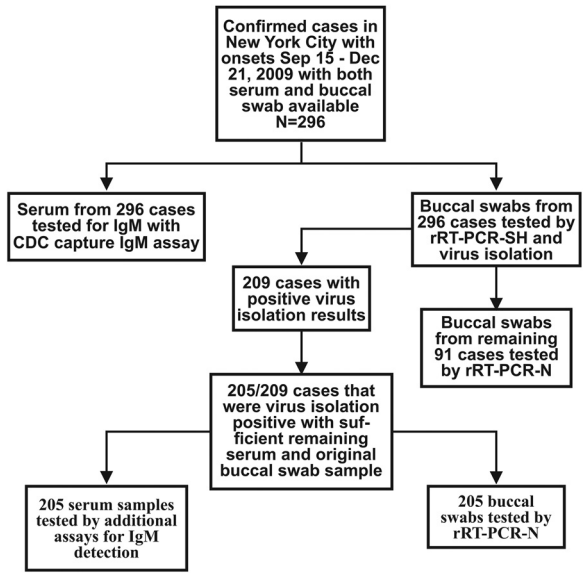

FIG 1 Study design for samples collected from cases identified during the New York City mumps outbreak with onsets of 15 September to 21 December 2009. Designation of subsets of samples used in the analyses as described in Materials and Methods and Results. rRT-PCR, real-time RT-PCR; SH, short hydrophobic gene; N, nucleoprotein gene.

Virus isolation using the Vero/hSLAM cell line (18) was performed with an aliquot of the buccal swab sample. Nucleotide sequences were determined from PCR products amplified from both original buccal swabs or from cell culture material as previously described (9, 10). Sequence analysis of the mumps SH gene and assignment of the mumps genotype were performed according to the methods described by Jin et al. (19).

Classification status as a confirmed case of mumps according to the CSTE case definition (Fig. 1; $n = 296$ cases) was considered the gold standard for evaluation of the performance of routine testing of CDC methods (capture IgM EIA and rRT-PCR-SH) and in the analysis of results by day of sample collection. Virus isolation was used as the gold standard for the comparisons of the two rRT-PCR assays and the 4 assays used for mumps IgM detection (Fig. 1; $n = 205$ cases).

The Fisher exact test was used to ascertain statistical significance for differences in the proportion of positive results between groups. The Cochran-Armitage test for linear trend was used for trend analysis of the proportion of positive results obtained versus timing of collection of samples.

RESULTS

Patients and samples. The vaccination status and demographic characteristics of the 296 case patients included in the New York City study were similar to those described for cases in the outbreak as a whole that included additional counties in New York and New Jersey (12, 13). Study cases ranged in age from 8 months to 84 years (median, 16 years), and 82% were male. A total of 155 (52.4%) cases were fully vaccinated with 2 or more documented doses of MMR; 25 (8.4%) had received 1 dose of MMR, 13 (4.4%) had received zero doses of MMR, and 103 (34.8%) had unknown vaccination status. Parotitis was the presenting symptom for 98% (291/296) of the study cases, and one case presented with orchitis. The remaining 4 cases were described as having facial (jaw or cheek) and/or neck pain either alone or accompanied by swelling.

Routine testing: CDC capture IgM EIA, IgG EIA, and rRT-PCR-SH. Serum samples from 296 confirmed mumps cases were

TABLE 1 Results obtained for serum samples tested using the CDC capture IgM EIA from 296 confirmed mumps cases, stratified by timing of serum collection and MMR vaccination status

No. of doses of MMR (n = samples)	No. (%) of serum samples that were IgM positive in MMR group/no. collected:		Total no. of samples positive in MMR group (%)
	0–2 days after onset[a]	≥3 days after onset[b]	
0 doses, n = 13	7/10 (70)	2/3 (67)	9/13 (69)
1 dose, n = 25	12/23 (52)	1/2 (50)	13/25 (52)
2 doses, n = 155	60/131 (46)	17/24 (71)	78/155 (50)
Unknown, n = 103	29/76 (38)	20/27 (74)	49/103 (48)
Total	108/240 (45)	40/56 (71)	148/296 (50)

[a] Median, 1.0 day; average, 0.78 days.
[b] Median, 5.0 days; average, 4.0 days.

TABLE 2 Results obtained with original buccal swab samples collected from 296 confirmed mumps cases were tested rRT-PCR using the mumps SH gene as the target, stratified by timing of buccal swab collection and MMR vaccination status

No. of doses of MMR (n = samples)	No. (%) of buccal samples that were rRT-PCR positive in MMR group/no. collected:		Total no. of samples positive in MMR group (%)
	0–2 days after onset[a]	≥3 days after onset[b]	
0 doses, n = 13	5/9 (55)	2/4 (50)	7/13 (54)
1 dose, n = 25	16/22 (73)	1/3 (33)	17/25 (68)
2 doses, n = 155	87/130 (67)	5/25 (20)	92/155 (59)
Unknown, n = 103	48/76 (63)	5/27 (18)	53/103 (51)
Total	156/237 (66)	13/59 (22)	169/296 (57)

[a] Median, 1.0 day; average, 0.8 days.
[b] Median, 4.0 days; average, 4.8 days.

tested using the CDC capture IgM EIA. Seven serum samples were indeterminate for IgM (data not shown); these were considered IgM negative for purposes of analysis. Table 1 shows the IgM results stratified by the vaccination status of the cases and grouped according to the timing of specimen collection. A higher proportion of cases that were unvaccinated were IgM positive (69%) compared to those with 1 or 2 doses of MMR vaccine or unknown vaccination status (48% to 52%). Overall, there were 148 (50%) serum samples with a positive result for IgM (Table 1). Most samples (81%, n = 240) were collected between 0 and 2 days after onset of symptoms; the remaining 56 (19%) serum samples were collected ≥3 days after onset. The capture IgM EIA was positive for 71% (40/56) of the specimens collected ≥3 days after onset of symptoms, whereas 45% (108/240) of specimens collected prior to day 3 were positive (P = 0.0006).

The serum samples were also tested for IgG as part of routine serologic testing performed at the CDC (data not shown). A negative result for IgG was obtained from only 9 (3%) of the serum samples; 2 samples were indeterminate for IgG. None of the serum samples tested positive for antibody to the Epstein-Barr virus (data not shown).

Virus isolation and rRT-PCR-SH were performed on buccal swabs from all 296 cases. Mumps virus was isolated from 209 (71%) of the 296 buccal swabs tested. Twenty-one of the cultures could not be completed due to contamination. The rRT-PCR-SH detected RNA in 169 (57%) buccal swab samples (Table 2). The rRT-PCR-SH results were stratified by MMR category and timing of buccal swab collection using the same intervals used for analysis of the serum samples. The proportion of buccal swabs collected at the earlier interval (80%) versus the later interval after onset (20%) was comparable to that for the serum samples. Overall, 156 (66%) of the buccal swabs collected 0 to 2 days after symptom onset were positive by rRT-PCR-SH compared with 13 (22%) of swabs collected ≥3 days after onset ($P < 0.0001$). No association was observed between rRT-PCR positivity and vaccination status.

The nucleotide sequences from 165 buccal swabs or virus isolates were analyzed. All of the sequences belonged to genotype G, except for 1 sequence that was determined to be genotype C. The genotype C sample was from a confirmed case that occurred in New York City in November 2009 but had no epidemiologic links to the Orthodox community.

Evaluation of IgM assays and rRT-PCR assays. Serum samples from 205 cases that were confirmed by virus isolation (Fig. 1) were included in the evaluation of the IgM assays. The serum samples were tested by four methods: two capture IgM assays, an indirect IgM EIA, and an IFA assay (Table 3). The two capture IgM assays performed best overall; the CDC capture IgM assay and the Microimmune assay detected IgM in 52% and 29% of the samples, respectively, compared with 12% by the indirect EIA and 15% using the IFA. In reading the IFA results, 31 serum samples were scored as indeterminate (based on consensus of 2 readers); these samples were considered IgM negative in the analysis.

The IgM results were stratified by MMR vaccination status of the cases (Table 3). The average interval from day of onset to serum collection did not vary among the four MMR groups (data not shown). Although the number of unvaccinated cases was low, the rate of IgM detection by all four assays was significantly greater among unvaccinated cases than cases vaccinated with ≥1dose of MMR vaccine. Concordant positive results were obtained for 8 of the 10 samples from unvaccinated cases using the four assays; the two capture IgM assays detected IgM in 9 of the 10 unvaccinated cases. The CDC capture IgM assay detected IgM in 51% of the samples from cases vaccinated with 2 doses of MMR vaccine,

TABLE 3 Comparison of mumps IgM detection using the CDC capture IgM EIA, two commercially available EIA kits, and an immunofluorescent antibody assay with serum samples from 205 cases that were confirmed by virus isolation

No. of doses of MMR	No. of samples positive/no. tested in MMR vaccination group (%)			
	CDC capture IgM[a]	Serion indirect EIA[b]	Microimmune capture EIA[a]	Bion fluorescent antibody[a]
0 doses	9/10 (90)	8/10 (80)	9/10 (90)	8/10 (80)
1 dose	9/17 (53)	2/17 (12)	8/17 (47)	2/17 (12)
2 doses	59/115 (51)	10/115 (9)	28/115 (24)	11/115 (9.5)
Unknown	29/63 (46)	5/63 (8)	15/63 (24)	9/63 (14)
Total	106/205 (52)	25/205 (12)	60/205 (29)	30/205 (15)

[a] Test performed at Centers for Disease Control and Prevention.
[b] Test performed at New York City Public Health Laboratory.

TABLE 4 Comparison of results obtained by testing original buccal swab samples from 205 cases that were confirmed by virus isolation by 2 rRT-PCR methods incorporating the gene encoding either SH or N as the target

No. of doses of MMR	No. of samples positive/no. tested by MMR group (%)[a]	
	rRT-PCR-SH	rRT-PCR-N
0 doses	7/10 (70)	9/10 (90)
1 dose	15/17 (88)	17/17 (100)
2 doses	88/115 (76)	109/115 (95)
Unknown	51/63 (81)	58/63 (92)
Total	161/205 (79)	193/205 (94)

[a] SH, short hydrophobic; N, nucleoprotein.

whereas positive IgM results were obtained for 9% to 24% of serum samples by the other three assays.

The ability to detect IgM improved among all assays when testing serum collected ≥3 days after symptom onset (data not shown). The highest proportion of positive samples among the 24 samples collected ≥3 days after onset was obtained by the Microimmune capture IgM assay (50%) and the CDC capture assay (79%). Of 8 serum samples collected ≥5 days after symptom onset, the CDC capture IgM EIA detected IgM in 8/8 (100%) of serum samples, the Microimmune assay detected IgM in 6/8 (75%), and both the Serion indirect EIA and the Bion IFA detected IgM in 3/8 (37.5%) of the serum samples (data not shown).

The rRT-PCR-N assay had a lower limit of detection of approximately 10 copies of mumps N gene RNA per sample. The specificity was evaluated by testing samples from sporadic cases of parotitis that were submitted for determination of alternative etiologic agents capable of causing parotitis. Of 38 samples that were identified as containing other etiologic agents (including Epstein-Barr virus, human herpesvirus 6B, human parainfluenza viruses 2 and 3), none were positive by the rRT-PCR-N assay (CDC, unpublished data).

The results from rRT-PCR using the two different target genes were compared by testing 205 buccal swab samples from cases that were confirmed by virus isolation (Table 4). Overall, the rRT-PCR-N assay detected mumps RNA in 94% (193/205) of the samples compared to 79% (161/205) using rRT-PCR-SH. No significant difference in the ability to detect mumps RNA by rRT-PCR was observed between vaccinated and unvaccinated cases.

The remaining 91 samples in the original study set of 296 cases were also tested using the rRT-PCR-N (Fig. 1). Only 4 samples in this group had been positive by virus isolation. The rRT-PCR-N detected mumps RNA in 8 buccal swab samples that had been negative by both virus isolation and by rRT-PCR-SH. However, the CDC capture IgM assay detected IgM in 33 of 75 cases that were negative by virus isolation, rRT-PCR-SH, and rRT-PCR-N. The remaining 42 cases were not laboratory confirmed (data not shown).

The results of testing all 296 cases by both rRT-PCR methods, along with the IgM results obtained with the CDC capture IgM assay, were analyzed based on the day of collection (Fig. 2). As the interval from onset to serum collection increased, the number of serum samples positive by the CDC capture IgM increased ($P = 0.0008$), while the number of buccal swab samples positive by rRT-PCR decreased with time ($P < 0.0001$). Among samples collected ≤2 days after symptom onset, rRT-PCR-N detected mumps RNA in 78% of the samples, and 66% of the samples were positive by rRT-PCR-SH; the proportion dropped to 41% and 22%, respectively, for samples collected ≥3 days after onset.

DISCUSSION

We made use of specimens from a large, well-characterized mumps outbreak to assess the sensitivity of diagnostic tests for mumps. Thus, we were able to evaluate the impact of vaccination status and the timing of specimen collection on the relative abilities of the standard diagnostic methods (IgM versus RNA detection) to confirm mumps infection as well as the performance of different IgM assays. The methodology for mumps rRT-PCR was improved by both the availability of a large number of clinical specimens from confirmed cases and the quality of the specimens, as demonstrated by the high proportion that were positive by virus isolation.

All serologic assays in the evaluation performed well to confirm the few cases in unvaccinated individuals, but the two capture IgM assays detected IgM in a higher percentage of the cases with a history of vaccination than the indirect EIA and IFA. Although an improvement in sensitivity was noted for all assays when serum was collected ≥3 days after onset, the effect of timing of collection did not alter the overall higher detection by the capture IgM assays. While the Microimmune capture IgM assay performed the best among the commercial assays, a higher percentage of IgM-positive samples was obtained with the CDC capture IgM assay. The apparent higher rate of detection of IgM by the CDC assay than the commercial capture IgM test could be due to selection of the serum specimens chosen to evaluate and validate the tests. Although avidity testing has revealed that persons with a secondary immune response are capable of eliciting an IgM response, the IgM response appears to be variable and generally not as reactive in EIAs as that observed in a typical primary response (20–23). Serum panels used to set the parameters for IgM detection (sensitivity) during the development of commercial assays are more likely to be collected from persons having a primary immune response and therefore have higher levels of IgM.

The timing of serum collection and the selection of the assay are both important when the majority of cases have been previ-

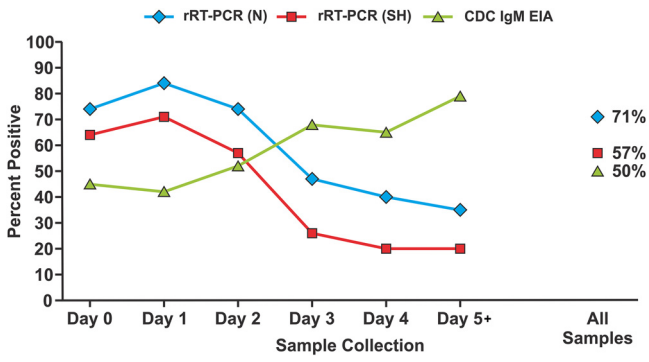

FIG 2 The percentage of positive results obtained from testing 296 confirmed mumps cases from New York City by day of sample collection after onset of symptoms. The serum samples were tested for presence of IgM using the CDC capture IgM EIA. The buccal swab samples were tested by 2 mumps rRT-PCR methods; either with the mumps nucleoprotein (N) gene as the target or with the short hydrophobic protein (SH) gene as the target.

ously vaccinated. During a mumps outbreak in Nova Scotia, collection of convalescent phase serum from 25 mumps cases yielded only one additional confirmation by IgM using an indirect EIA (23). While use of a capture IgM EIA may have improved IgM detection in that situation, individuals who have previously responded to mumps vaccination may not produce mumps-specific IgM, or the levels present may be undetectable by any assay.

The outbreak in New York City also provided the opportunity to discern differences in sensitivity in rRT-PCR using N versus SH as the target gene. Among 296 cases, rRT-PCR-N increased RNA detection by 14% compared with that of rRT-PCR-SH. There were positive results for 209 (71%) samples using the rRT-PCR-N. An equal number of samples were positive by virus isolation. However, 12 samples that were negative by virus isolation were positive by rRT-PCR-N, and 12 that were negative by rRT-PCR-N were positive by virus isolation. Buccal swab samples from 69 additional cases of mumps in New York City from January to March 2010 continued to be tested by both rRT-PCR-N and virus isolation. Concordant results were obtained by rRT-PCR-N and virus isolation with the exception of 4 samples that were again equally divided for positive results. Therefore, in most cases, rRT-PCR should provide sensitivity equivalent to viral culture and with much more rapid results.

A higher rate of case confirmation was obtained in the New York City outbreak by both IgM detection and by rRT-PCR than had been described following mumps outbreaks in 2006 using the same methods at the CDC (9, 10). Mumps RNA was detected in 57% of the cases in New York City using rRT-PCR-SH, an increase of over 25% compared to that obtained from 2 outbreaks in 2006 (9, 10). The proportion of cases that were positive by the CDC capture IgM EIA was also higher in this study (50%) than that of the outbreaks in 2006 (13% to 14%), even though the proportions of vaccinated cases were comparable (7–10, 12, 13). Consistent with previous reports, the sensitivity obtained by rRT-PCR was higher than that obtained for IgM detection. However, this study demonstrated that the timing of sample collection and the type of IgM assay utilized are important factors.

There are several possible explanations for the improved rates of case confirmation in the New York City outbreak compared to those in the previous reports in which the same laboratory testing methods were used. The number of non-mumps cases or cases with mild symptoms included in the evaluation of the outbreaks in 2006 may have been much higher. Whereas the cases in New York City were nearly all described as having parotitis, it was not unusual for symptoms to be recorded as jaw or facial soreness or swelling during the outbreaks in 2006 (8) (CDC, unpublished data). The intensity of exposure among the boys who attended Jewish schools was suggested to have played a large role in the New York City outbreak (13). Lengthy study sessions involving face-to-face recitations provided opportunities for repeated and efficient transmission of infectious droplets. The clinical presentation of mumps among previously vaccinated persons may vary depending on the dose of virus transmitted. One could speculate that confirmation of mumps by RNA or IgM detection is more challenging among milder cases of mumps. Finally, adherence to recommendations for sample collection and transportation during the New York City outbreak may have contributed to the higher rate of laboratory confirmation observed in this study.

Our study has limitations. The small number of unvaccinated cases in the study and the unknown proportion of primary vaccine failures among the vaccinated cases may have limited our ability to ascertain the extent of variation in the performance of the assays based on vaccination status. Though the vaccinated cases were presumed to be the result of secondary vaccine failure, IgG avidity testing was not conducted. However, whereas all of the IgM assays detected IgM in 80% to 90% of the unvaccinated cases, IgM detection dropped to 10% to 51% among the vaccinated cases. While there may have been primary vaccine failures among the vaccinated cases, the lower sensitivity for IgM suggests that a majority of the vaccinated cases had a secondary type immune response. However, if the proportion of primary vaccine failures was higher among the New York City cases than among the vaccinated cases tested in the 2006 outbreaks, the difference could explain why we obtained higher rates of IgM and RNA positivity in this study.

The data from our study indicated that RNA detection by rRT-PCR decreases after 2 days after onset regardless of vaccination status. The low proportion of unvaccinated cases in this study may have limited our ability to detect an effect of vaccination status on the performance of rRT-PCR. The importance of early collection of samples for successful detection of mumps RNA in highly vaccinated populations has been reported previously and was speculated to be due to more rapid clearance of virus by persons with residual antibody from vaccination (9, 10).

The confirmed cases of mumps that were negative by all laboratory tests ($n = 42$) may have been misclassified despite epidemiologic links to the outbreak. Inclusion of non-mumps cases would result in lower sensitivity of the diagnostic assays. However, the localized nature of the New York City outbreak (13) and the improvement in PCR and/or IgM detection compared to the results obtained in 2006 suggest that most, if not all, of the cases were true cases of mumps.

It is recommended that providers collect both buccal swab specimens and serum samples when mumps infection is suspected. Although it was demonstrated that rRT-PCR was more sensitive than IgM detection, particularly when samples were collected within 2 days of symptom onset, the quality of virologic samples is variable, and delays in obtaining specimens are often unavoidable. Provider education regarding the proper collection techniques, transport methods, and recommended assays may improve the ability to confirm mumps infection among vaccinated populations.

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The findings and conclusions of this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention

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