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Confirmation of Rubella within 4 Days of Rash Onset: Comparison of Rubella Virus RNA Detection in Oral Fluid with Immunoglobulin M Detection in Serum or Oral Fluid▽

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Rubella virus infection is typically diagnosed by the identification of rubella virus-specific immunoglobulin M (IgM) antibodies in serum, but approximately 50% of serum samples from rubella cases collected on the day of rash onset are negative for rubella virus-specific IgM. The ability to detect IgM in sera and oral fluids was compared with the ability to detect rubella virus RNA in oral fluids by reverse transcription-PCR (RT-PCR) by using paired samples taken within the first 4 days after rash onset from suspected rubella cases during an outbreak in Perú. Sera were tested for IgM by both indirect and capture enzyme immunoassays (EIAs), and oral fluids were tested for IgM by a capture EIA. Tests for IgM in serum were more sensitive for the confirmation of rubella than the test for IgM in oral fluid during the 4 days after rash onset. RT-PCR confirmed more suspected cases than serum IgM tests on days 1 and 2 after rash onset. The methods confirmed approximately the same number of cases on days 3 and 4 after rash onset. However, a few cases were detected by serum IgM tests but not by RT-PCR even on the day of rash onset. Nine RT-PCR-positive oral fluid specimens were shown to contain rubella virus sequences of genotype 1C. In summary, RT-PCR testing of oral fluid confirmed more rubella cases than IgM testing of either serum or oral fluid samples collected in the first 2 days after rash onset; the maximum number of confirmations of rubella cases was obtained by combining RT-PCR and serology testing.

Symptomatic rubella is characterized by a mild fever and a maculopapular rash of short duration. The clinical diagnosis of rubella is unreliable, and many rash illnesses, such as those caused by measles virus and parvovirus B19, mimic rubella (2). Therefore, laboratory confirmation is essential for the diagnosis of rubella and is typically done by testing serum samples for rubella virus (RV)-specific immunoglobulin M (IgM) antibodies. Serum IgM and IgG responses to RV develop rapidly in the first few days after the onset of rash. However, approximately 50% of samples collected on the day of rash onset test negative for RV-specific IgM antibodies (1, 9, 17). Often, only a single serum sample taken near the time of rash onset is available, resulting in the lack of serologic confirmation of many rubella cases. Thus, the development of a rapid laboratory diagnostic tool for the confirmation of rubella within the first few days of symptom onset would improve the ability to confirm rubella.

The isolation of virus in cell culture or the detection of viral RNA by reverse transcription-PCR (RT-PCR) also provides reliable evidence of RV infection (26). Unfortunately, blood is not a good sample for use for the detection of RV, because the highest viral titers in blood typically occur before the onset of rash and virus is undetectable in blood by 2 days after rash onset (6). The virus titer in throat swabs, however, usually reaches a peak titer on the day of rash onset and the titers in throat swabs decline more slowly than those in blood, so that virus can be detected for up to 5 to 7 days after rash onset (6). Several RT-PCR assays for the detection of the RV genome in clinical samples have been described (3, 7, 15, 16, 20, 25). Templates for the determination of viral sequences for molecular epidemiology can also be made by using RT-PCR.

The use of alternative specimens could help reduce the obstacles to specimen collection, storage, and transport in the field (22). Oral fluid (OF), which is collected by rubbing an absorptive device between the gum and the cheek, can be obtained by a method that is relatively noninvasive, is easier to obtain than blood, and has the advantage that it can be used for both RV-specific antibody detection and RV genome detection (12, 19, 20). Currently, in the United Kingdom, OF samples from notified clinically diagnosed cases are collected between 1 and 6 weeks after the onset of symptoms and are transported by mail to the Central Public Health Laboratory, where they are tested for specific antibody and viral RNA by RT-PCR. By the use of this strategy, specimens from 54.6% of rubella notifications from 1995 through 2001 were obtained for laboratory testing and specimens from 12.7% of the rubella notifications were confirmed to represent rubella cases (20, 21).

The current study compared the detection of RV-specific IgM in serum and OF to detection of RV RNA by RT-PCR in OF specimens collected from health care provider-diagnosed

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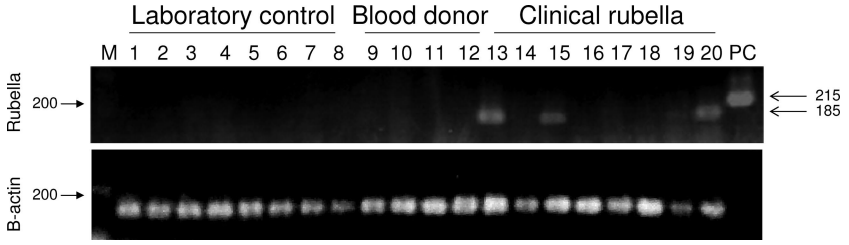

FIG. 1. Agarose gel of products obtained by conventional RT-PCR of representative samples from controls and suspected rubella cases. (Top panel) Products obtained by RT-PCR for the 185-bp amplicon using transport medium (negative laboratory controls) (lanes 1 to 8), samples from healthy blood donors (negative patient controls) (lanes 9 to 12), or samples from suspected rubella cases (lanes 13 to 20). The samples in lanes 13, 15, and 20 are positive for rubella RNA. Lane PC, positive control amplified from the RV RNA transcript containing a 30-nt insertion; lane M, DNA marker. (Bottom panel) Results for the controls and suspected rubella cases in which the samples were amplified with β-actin primers that produce a 150-bp fragment. The results for all blood donors and suspected rubella cases shown were positive for this control for RNA integrity. The eight negative laboratory control extracts from transport medium (lanes 1 to 8) were also positive for β-actin because the medium contained fetal bovine serum.

rubella patients during the first 4 days after rash onset. The samples were collected during a large rubella outbreak in Perú in 2004 and 2005 (10).

MATERIALS AND METHODS

Study population and sample collection. The specific methodology, including ethics approval, has been described previously (10). Briefly, health care workers in five zones in Perú enrolled persons ≥8 months of age who presented to local health care centers with suspected rubella (fever and rash) within 28 days of rash onset. Individuals were excluded if they had been vaccinated within 8 weeks of presentation, were pregnant, or had chronic diseases. Healthy adult blood donors in the city of Lima were also enrolled in April and May 2005 as control persons; no outbreak of rubella was known to have occurred in this population during this time period. Adults were chosen for use as controls because it was reasonable to assume that their specimens would not differ significantly from those of younger persons in the tests used in the present study. Blood for serum preparations was collected by venipuncture. The OF samples were collected with an Oracol device (Malvern Medical, Worcester, United Kingdom), as recommended by the manufacturer.

Sample processing. Blood and OF specimens were transported on wet ice to the Peruvian Ministry of Health's National Reference Laboratory for Measles/Rubella, and blood samples were processed as described previously (10). OF specimens were stored unprocessed at −20°C for up to 4 months. At the end of the collection phase of the study, all samples were shipped to the Centers for Disease Control and Prevention in Atlanta, GA, on dry ice and were stored at −70°C. OFs were eluted from the Oracol devices by the addition of 1 ml of transport medium (phosphate-buffered saline containing 10% fetal bovine serum, 0.02% Tween 20, 0.5% gentamicin, and 0.2% amphotericin B [Fungizone]) (4), followed by incubation at 4°C overnight. The samples were then centrifuged at 2,000 rpm for 5 min. Most of the supernatant was aliquoted and stored at −70°C. The cell pellet was resuspended in approximately 200 μl of residual supernatant plus 200 μl of transport medium, and the mixture was stored at −70°C.

RV-specific IgM antibodies in serum and oral fluid. Serum samples were tested for RV-specific IgM antibodies with a Dade Behring Enzygnost (DBE) anti-RV IgM antibody enzyme immunoassay (EIA) kit (Marburg, Germany) and a Microimmune (MI) RV IgM capture EIA (Middlesex, United Kingdom), according to the manufacturers' instructions. Eluted OF supernatants were tested for RV-specific IgM antibodies by the MI rubella IgM capture EIA according to the manufacturer's instructions. Unlike serum samples, OF samples were not diluted prior to addition to the test plate. According to the instructions provided with either kit, samples yielding equivocal results were retested and were classified as positive, negative, or equivocal depending on the outcome of the second test, with one exception. One OF sample had an insufficient volume for retesting, and the initial equivocal result was retained.

RNA extraction and conventional RT-PCR. RNA was extracted from 140 μl of the resuspended cell pellet of OF from both suspected rubella cases and blood donors by using a QiaAmpViral RNA minikit (Qiagen, Valencia, CA), according to the manufacturer's instructions. For negative laboratory controls, RNA was extracted from 140 μl of the transport medium. To control for cross-contamination from positive specimens, specimens from negative laboratory controls were always run in parallel with specimens from suspected cases (Fig. 1). RT-PCR for the generation of a 185-bp amplicon was performed with a SuperScript OneStep RT-PCR kit (Invitrogen, Carlsbad, CA), as described previously (25), except that the number of amplification cycles was increased from 35 to 40. In addition, for many of the OF-derived RNAs (the first 45 samples processed and then 1 sample in every batch of 10), amplification of a 150-bp region of the β-actin gene was performed as a control for the quality of the RNA extractions. To allow possible contamination of RV control RNA to be identified, a genetically engineered RV RNA transcript was used as a positive control for the RT-PCR. This RNA transcript, which was transcribed in vitro from a cDNA construct encoding the entire RV structural protein-coding region, contained an insertion of 30 nucleotides (nts) of nonviral sequence within the region amplified by the 185-bp amplicon primers. Five microliters of each RT-PCR product was visualized on a 1.5% agarose gel with ethidium bromide.

Real-time RT-PCR. The real-time RT-PCRs were done with the same 185-bp amplicon primers and kit described above. The TaqMan probe, designed by the use of Applied Biosystems (AB; Foster City, CA) Primer Express software, contained RV nts 8835 to 8854 (5′-AGGTCCAGGTCCCGCCCGAC). The probe was synthesized at the Biotechnology Core Facility at the Centers for Disease Control and Prevention and was labeled at the 5′ end with the reporter molecule 6-carboxyfluorescein and at the 3′ end with the quencher Blackhole Quencher 1 (Biosearch Technologies, Novato, CA). The assay was performed in triplicate with 25-μl reaction mixtures containing reaction buffer, 10 U of RNase inhibitor, 0.5 μl of a Superscript-*Taq* enzyme mixture, 0.2 μM of each primer, 0.1 μM of the labeled probe, 0.15 mM of $MgSO_4$, and 2.5 μl of RNA. The thermal cycling was carried out with an AB Prism 7900HT thermal cycler with 48°C for 30 min, 95°C for 5 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. The presence of intact RNA in the samples was confirmed with primers specific for RNase P RNA (8). Four positive controls containing known copy numbers of transcribed RV RNA were run on each plate as quantification standards. A reaction mixture containing water as the template was run on each plate as a negative control. The data were analyzed with SDS software (version 2.1; AB). Test samples were considered positive if amplification was seen in at least two of the triplicate reactions and by use of the signal for all wells in cycles 3 to 15 as the background signal. The sensitivities of the tests reported here were at least 12 copies of RV RNA.

Nested-set RT-PCR for genotyping. Two pairs of specific primers were chosen for use in the nested-set RT-PCR to amplify an 876-nt region of the RV E1-coding region, which encompassed the 739-nt region recommended by WHO for use for RV genotyping (23). The first-round primers were 8656F (5′-CCCCACCGACACCGTGATGAG) and Rub3′ (5′-TTTTTTTTT TTTTTTTT TCTATACAGCAACAGGTGC). The second-round primers were 8669F (5′-GTGATGAGCGTGTTCGCCCTT) and 9549R (5′-TGGTGTGTGTGCCA TAC). RT-PCR was performed with an Access RT-PCR system kit (Promega, Madison, WI). For the first-round RT-PCR, 5 μl of RNA was added to 45-μl reaction mixtures containing reaction buffer, a 0.2 mM deoxynucleoside triphosphate mixture, 1 mM $MgSO_4$, 1 μM each of the first-round primers, 0.1 U avian myeloblastosis virus reverse transcriptase, 0.1 U *Tfl* DNA polymerase, and 1 M betaine. Betaine was added to the kit components to facilitate amplification of the RV G+C-rich RNA (14). Thermal cycling was

TABLE 1. Summary of RV-specific IgM results for serum and OF sample pairs for persons with suspected rubella

MI EIA result with OF	No. (%) of specimens with the following DBE EIA result with serum:			
	Positive	Equivocal	Negative	Total
Positive	79 (35)	3 (1)	0	82 (36)
Equivocal	22 (10)	4 (2)	2 (1)	28 (13)
Negative	44 (19)	20 (9)	51 (23)	115 (51)
Total	145 (64)	27 (12)	53 (24)	225

as follows: 45°C for 45 min, followed by 94°C for 2 min and then 40 cycles of 94°C for 30 s, 60°C for 1 min, and 68°C for 2 min. For the second round, 3 μl of the first-round product was used as the template in the PCR mixture, as described above, except that the avian myeloblastosis virus reverse transcriptase was eliminated and the 45°C segment of cycling was removed. This protocol usually allows the genotypes of the RVs present to be determined from specimens which are positive by conventional RT-PCR (e.g., 9 of 11 specimens in the present study [see below]).

Sequencing and phylogenetic analysis. DNAs from nested-set RT-PCR-positive reactions were purified with a Wizard SV Gel and PCR cleanup system (Promega). The 739-nt sequences of the standard window used for the genotyping of RVs were determined bidirectionally with an AB Prism BigDye Terminator cycle sequencing ready reaction kit and a 3100 DNA sequencer (AB). Phylogenetic analysis was done with the 32 WHO reference virus sequences (24) and the heuristic tree search algorithm using the maximum-parsimony criterion of the PAUP search program from the Genetics Computer Group (version 10.3; Accelrys, San Diego, CA).

Nucleotide sequence accession numbers. The nucleotide sequences of the RVs in the nine samples found to contain RV of genotype 1C were submitted to the GenBank database and can be found under accession numbers EU622498 to EU622506.

RESULTS

Paired serum and OF samples were collected from a total of 225 persons with suspected rubella within the first 4 days after rash onset. Samples were collected at later time points, but there were insufficient numbers (38 samples spread over a 24-day period) for reliable comparisons to be made. Paired samples that served as controls were available from 99 blood donors. The median age of the 225 enrollees with suspected rubella was 7.6 years, and the median age of the 99 blood donors was 27 years. Forty-six percent (103/225) of the enrollees with suspected rubella were male, and 87% (86/99) of the blood donors were male.

Antibody detection in serum and OF. The comparison of the serum DBE EIA and the OF MI EIA results for samples from the 225 suspected rubella patients are shown in Table 1. The results of the two tests were 60% concordant (134/225). Comparisons of the ability to detect the presence of IgM in OF and serum depend upon the interpretation of the equivocal results. Comparison of the results obtained with serum and OF showed that for the serum samples which were IgM positive by the DBE EIA, 63% (108/172; 95% confidence interval [CI], 55% to 70%) of the paired OF samples were IgM positive by the MI EIA if the samples with equivocal results were included with those with positive results, 65% (79/123; 95% CI, 55% to 73%) were positive if the samples with equivocal results were omitted, and 54% (79/145; 95% CI, 46% to 63%) were positive if the results for samples with equivocal results were considered negative. For serum samples that were IgM negative by the DBE EIA, 100% (51/51) of the paired OF samples were also IgM negative by the MI EIA (95% CI, 91% to 100%) if the samples with equivocal results were omitted, but the rates of concordance dropped to 96% (51/53; 95% CI, 86% to 99%) if the samples with equivocal results were included with the samples with positive results and 96% (77/80; 95% CI, 89% to 99%) if the samples with equivocal results were considered negative. Among the 99 serum samples from blood donors, 1 had an equivocal result by both the DBE and the MI EIAs, 2 had equivocal results by the DBE EIA only, and 1 had a positive result by the MI EIA only. All of the 99 donor OF samples were negative by the MI EIA. The blood donor control results allow the rates of false positivity to be estimated to be no more than 1% for all of the assays used in this study. Serum samples from persons suspected of having rubella were also tested for IgM antibodies by the MI EIA, and the number

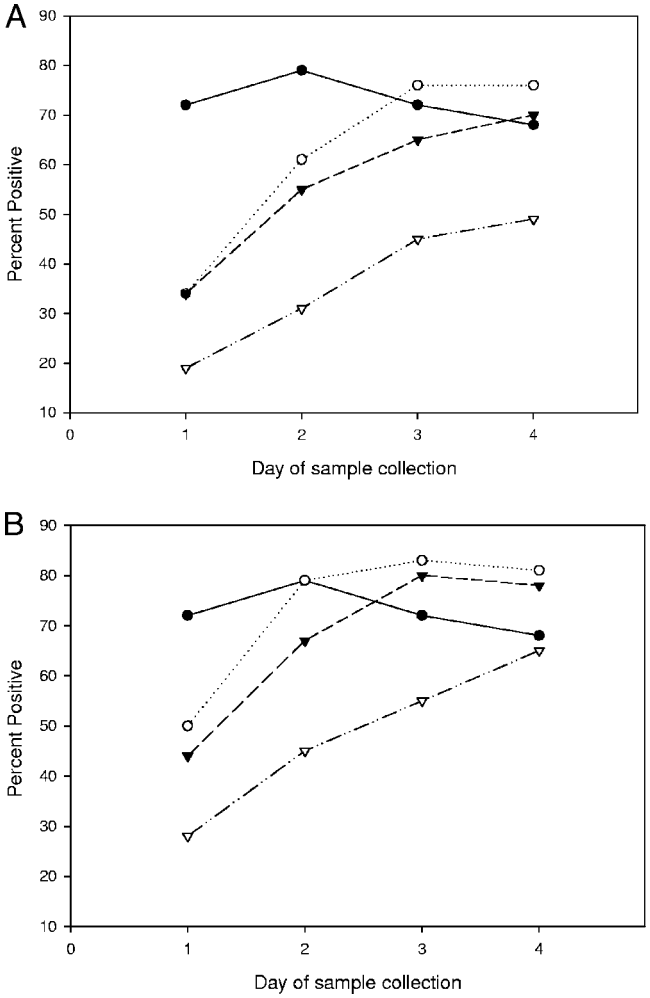

FIG. 2. Percentage of suspected rubella cases, as confirmed by four tests. The results obtained by RT-PCR (a combination of conventional and real-time RT-PCR results) of OF (●), analysis of OF for IgM antibodies by the MI EIA (Δ), analysis of serum for IgM antibodies by the MI EIA (▶), and analysis of serum for IgM by the DBE EIA (○) during the first 4 days after rash onset are shown. (A) Percent positive when the results for samples with equivocal results are not included; (B) percent positive when the results for samples with equivocal results are included as positive results.

TABLE 2. Comparison of IgM detection in serum by DBE EIA with RNA detection in OF by RT-PCR

Day	No. of sample pairs	No. (%) of sample pairs with the followingOF RT-PCR result and the indicated DBE EIA result with serum:							
		OF RT-PCR positive				OF RT-PCR negative			
		Positive	Equivocal	Negative	Total	Positive	Equivocal	Negative	Total
1[a]	32	8	5	10	23 (10)	3	0	6	9 (4)
2	85	43	13	11	67 (30)	9	2	7	18 (8)
3	71	41	5	5	51 (23)	13	0	7	20 (9)
4	37	22	2	1	25 (11)	6	0	6	12 (5)
Total for days 1–4	225	114 (51)	25 (11)	27 (12)	166 (74)	31 (14)	2 (1)	26 (12)	59 (26)

[a] Day 1 was the day of rash onset.

of confirmed rubella cases was only slightly lower than the number obtained by the DBE EIA (Fig. 2).

RT-PCR. The conventional RT-PCR for RV RNA was positive for the 185-bp amplicon with samples from 68% (154/225) of suspected rubella patients and was negative with samples from all 99 blood donors. All laboratory control RNAs extracted from transport media were negative by RT-PCR. The RT-PCR for the 150-nt β-actin fragment was positive for all samples tested, confirming the integrity of the RNA (Fig. 1). The real-time RT-PCR was performed with samples from the 71 suspected rubella patients which were negative by the conventional RT-PCR, 20 samples from the 71 suspected rubella patients which were positive, and 96 of the blood donor samples. All 20 randomly chosen samples that were positive by the conventional RT-PCR were positive by the real-time RT-PCR, and all the blood donor samples were negative. Seventeen percent (12/71) of the conventional RT-PCR-negative specimens from suspected rubella patients were positive by the real-time RT-PCR. Thus, the overall positive detection rate for RT-PCR (either technique) was 74% (166/225).

Comparison of OF RT-PCR and IgM serology. The results of RT-PCR and antibody testing for suspected cases were compared by day of specimen collection (Fig. 2). Figure 2A summarizes the positive results. On day 1, RT-PCR confirmed significantly more suspected cases than detection of IgM antibodies in serum by the DBE EIA, detection of IgM antibodies in serum by the MI EIA, or detection of IgM antibodies in OF by the MI EIA (72% [28/32; 95% CI, 56% to 87%] for RT-PCR versus 34% [11/32; 95% CI, 18% to 51%], 34% [11/32; [95% CI, 18% to 51%], and 19% [6/32; 95% CI, 5% to 32%] for the three serology tests, respectively). On days 2, 3, and 4, the percentages of suspected cases which were IgM positive rose, while the percentages which were RT-PCR positive remained relatively constant. Nevertheless, RT-PCR still confirmed significantly more suspected cases than any IgM test on day 2, but only at the 90% CI level. The results of the two serum antibody tests were similar, although the percentage of suspected cases which were MI EIA positive was lower than the percentage of suspected cases which were DBE EIA positive on days 2, 3, and 4. The percentage of suspected rubella cases which were confirmed by MI EIA testing of OF was lower than the percentage of suspected rubella cases which were confirmed by either serum IgM test for days 1 to 4.

There were very few equivocal results for the large control population of blood donors enrolled (e.g., 3/99 [3%] by the DBE EIA) and a larger percentage of equivocal results among suspected rubella cases (e.g., 27/225 [12%] by the DBE EIA), supporting the inclusion of the samples with equivocal results with the samples with positive results, as has been done previously with dried blood spots and serum (10, 11). The results obtained when the samples with equivocal IgM test results were included with those with positive IgM test results are shown in Fig. 2B. DBE EIA testing of serum and RT-PCR testing of OF detected the same numbers of rubella cases on day 2. The only IgM test results significantly different at the 95% CI level from the OF RT-PCR results were those from the OF MI EIA on day 1 and day 2 (for day 2, 79% for RT-PCR [67/85; 95% CI, 70% to 88%] versus 44% for OF MI EIA [38/85; 95% CI, 34% to 55%].

The DBE EIA results, which gave the highest number of positive results for serum, are compared with the results of RNA detection in OF by RT-PCR in Table 2. Overall, 12% (27/225) of the suspected rubella cases were RT-PCR positive but IgM negative; the number of suspected rubella cases confirmed by RT-PCR but missed by antibody testing ranged from 31% (10/32) on day 1 to 3% (1/37) on day 4. Fourteen percent (31/225) of the sample pairs were RT-PCR negative but IgM antibody positive; the number of suspected rubella cases confirmed by IgM testing but missed by RT-PCR ranged from 9% (3/32) on day 1 to 18% (13/71) on day 3. Interestingly, almost all of the patients who had equivocal results by IgM testing were also OF RT-PCR positive (25/27 [93%]). Overall, 12% (26/225) of the samples were negative by both RT-PCR and IgM antibody testing.

Genotyping. Eleven OF samples from suspected rubella cases which tested positive for the 185-bp amplicon by RT-PCR were selected for genotyping analysis. The samples were obtained in November and December of 2004 and January and February of 2005 and from four of the five study zones (one zone, Huánuco, had no RT-PCR-positive samples). A 739-nt sequence was obtained from nine samples, and all samples were found to contain RV of genotype 1C. They varied from each other by at most 2 nucleotides (Fig. 3).

DISCUSSION

This study was designed to determine whether the use of OF as an alternative sample to serum could improve the laboratory confirmation of rubella in the first 4 days after the onset of the rash. The detection of viral RNA was found to confirm the

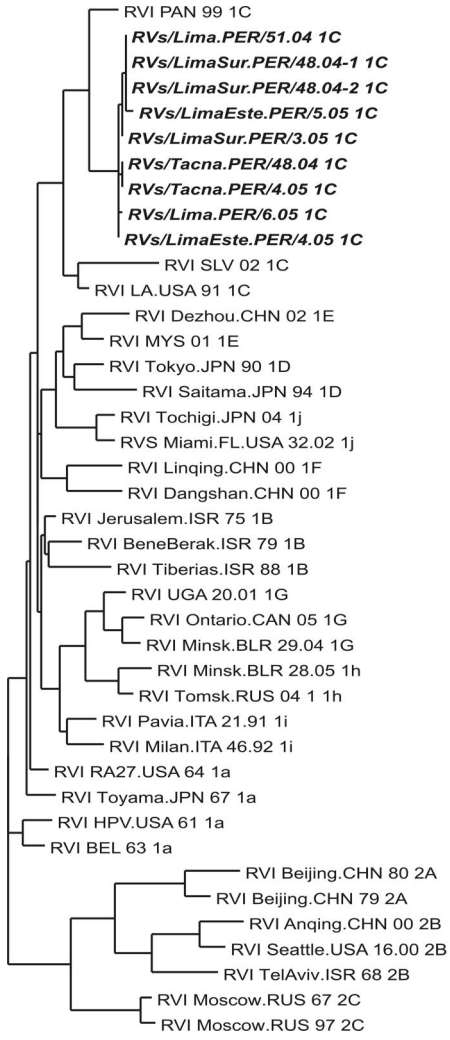

FIG. 3. Phylogenetic analysis of 739-nt sequences from the E1-coding region of wild-type RVs. The unrooted tree was made by using the maximum-parsimony criterion of the PAUP search program (version 10.3; Genetics Computer Group). The tree was constructed by using the 739-nt sequences from positions 8731 to 9469 and contains the 32 WHO accepted reference strains and the 9 sequences from Perú (in boldface and italics). All reference viruses grouped as expected, which is the primary criterion for a valid RV genotyping analysis result.

most cases, and the detection of IgM antibodies in OF was found to confirm fewer cases than the detection of either RNA in OF or IgM antibodies in serum.

The detection of IgM antibodies in OF by the MI EIA is less sensitive than the detection of IgM antibodies in serum by either the MI EIA or the DBE EIA. The nearly equivalent performance characteristics of the DBE and MI kits with sera indicated that the lower rate of detection of IgM antibodies in OF by the MI EIA was due to differences between OF and serum rather than differences between the kits. The inclusion of equivocal results with positive results increased the sensitivity by 10% for all IgM methods, but the rate of confirmation of the cases by the detection of IgM in OF by the MI EIA was still lower than that obtained when serum was tested by the MI EIA.

Other studies of the detection of IgM antibodies in OF taken at later time points after disease onset have found the rates of positivity for IgM antibodies in serum and OF to be similar. In one study (13), IgM antibodies were detected in the OF of 90% of patients with serum IgM antibodies for specimens collected between 7 and 42 days after disease onset. In another report (18), IgM antibodies were detected in the OF of 95.5% of patients with serum IgM antibodies for specimens taken between 10 to 14 days after disease onset and in 100% of 111 cases with congenital rubella syndrome. The current study extends these results to OF specimens taken in the first 4 days after rash onset, and the rates of detection at these times were low. Indeed, the instruction manual accompanying the MI EIA kit notes that the use of samples taken less than 7 days from the time of rash onset for the detection of antibodies is not optimal. Unfortunately, in outbreak situations in many countries, for an illness such as rubella that presents with a mild rash, patient contact with health care providers will usually be on or near the day of onset and the rates of IgM antibody detection in OF will be even lower than the rates of IgM antibody detection in serum in these situations.

The conventional RT-PCR assay used in this study was very sensitive, detecting RV RNA in 93% (154/166) of the OF samples in which RV RNA was detected by the more sensitive real-time RT-PCR. Although the sensitivity of the real-time RT-PCR assay was determined to be 10-fold higher than that of the conventional RT-PCR when RV RNA transcribed in vitro was used (data not shown), the real-time assay was able to increase the rate of detection of RV-positive OF samples by only 6% (from 68% to 74%). This is an encouraging result, as many more laboratories have conventional RT-PCR capabilities than the more expensive real-time equipment.

The detection of RV RNA in OF was more sensitive than the detection of IgM antibodies in either serum or OF for the confirmation of rubella by using samples collected early after rash onset. With samples collected on the day of rash onset, RT-PCR confirmed about 2.1 times more rubella cases (1.6 times if the samples with equivocal results were considered positive) than tests for the detection of IgM antibodies in serum. The sensitivity of RT-PCR remained higher with samples collected on day 2 after rash onset. In the first few days after rash onset, the use of RT-PCR RNA detection instead of IgM antibody detection would improve the rate of confirmation of rubella cases. With samples collected on the day of rash onset, however, RT-PCR did have some limitations. In this study, 9% (3/32) of samples collected on the day of rash onset were IgM positive but RT-PCR negative. There are several possible reasons for this, including the use of poor procedures for sample collection and the degradation of RNA due to the transport or storage conditions used.

Clearly, the presence of RV RNA and the presence of antibodies (IgM and IgG) to RV are not entirely independent of one another. For example, a very robust immune response would likely be correlated with the presence of smaller amounts of RNA due to viral clearance. The very small proportion of serum samples with equivocal results for IgM antibodies in RT-PCR-negative persons in the present study (Table 2) may be the result of a robust immune response in these persons. Furthermore, a result supporting this hypothesis is the fact that the number of OF samples

from the RT-PCR-negative group of patients with equivocal results for IgM antibody detection was also one-third lower than the number of OF samples from the RT-PCR-positive group of patients with equivocal results for IgM antibody detection (data not shown). Conversely, for the RT-PCR-positive group, the higher proportion of serum (and OF) samples with equivocal results for IgM antibody detection may be the result of a less robust immune response in these patients at the time of specimen collection.

One limitation of this study was the inability to obtain the convalescent-phase serum samples from the suspected rubella cases needed for the confirmation of RV infections. The availability of second serum samples collected 2 weeks after rash onset, when nearly all cases should be IgM antibody positive, would have allowed the nonrubella cases to be excluded. The collection of such a second serum sample was part of the study design. However, all participants declined to return for collection of a convalescent-phase serum sample. Since the enrollment was based on clinically diagnosed rubella and the clinical diagnosis is unreliable even during an outbreak, many of the 11.6% of enrollees who were both negative for serum IgM antibodies by the DBE EIA and negative for RV RNA in OF by RT-PCR were probably not rubella cases. Nevertheless, the lack of return visits in this study indicates that OF may be a better specimen than serum for use in RT-PCR for case-based surveillance in many countries seeking to eliminate rubella (e.g., Perú), where the mildness of the disease means that patients will not voluntarily return even a few days after disease onset. Of course, the availability and cost of RT-PCR versus those of serum IgM antibody testing may be other factors related to decisions about the best laboratory test to be used for a particular surveillance system.

The WHO Measles/Rubella LabNet has recommended the collection of RV genotype data to support rubella control programs globally (5). The successful genotyping of the viruses by the use of OF specimens reported here is consistent with a previous report of rubella genotyping by the use of OF specimens (20) and confirmed that the genetic characterization of RVs by the use of OF specimens is feasible in an outbreak situation. The viral sequences of the 739-nt sequence of the E1-coding region recommended for use by WHO were obtained from samples collected at several locations and time points during the outbreak, allowing confirmation that the outbreak was initiated by a genotype 1C virus and that this virus was present throughout the outbreak.

Information on the molecular epidemiology of measles has been very valuable in supporting efforts to achieve the elimination of measles in the Americas (5). Unfortunately, it has been difficult to develop a robust database of RV sequences to support the elimination of rubella in the Americas. The collection of OF for routine surveillance for rubella would provide many more opportunities to obtain the sequences of the RVs circulating in the Americas.

This study demonstrated that the use of OF for the detection of RV RNA is a sensitive method of laboratory confirmation of the diagnosis of RV infections in the first few days after rash onset. Laboratory testing for rubella by use of a combination of RT-PCR and IgM serology would allow the rapid confirmation of most cases in the first few days after the appearance of disease symptoms. For samples collected 3 or more days after rash onset, IgM serology alone is likely to be sufficient for the confirmation of rubella.

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We report no conflicts of interest.

The findings and conclusions in this article are those of the individual authors and do not necessarily reflect the views of the U.S. Department of Health and Human Services. The use of trade names and commercial sources is for identification only and does not imply endorsement by the U.S. Department of Health and Human Services.

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