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Use of PCR for Prenatal and Postnatal Diagnosis of Congenital Rubella

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A reverse transcription-nested PCR assay (RT-PCR) was evaluated for diagnosis of congenitally acquired rubella in utero and during infancy. RT-PCR was compared with virus isolation for retrospective detection of rubella virus in placental and fetal tissues obtained after termination of pregnancy following primary rubella or rubella virus reinfection. Concordant results were obtained for 85% of samples; rubella virus RNA was detected by RT-PCR alone in four samples, and rubella virus was detected by isolation alone in two samples. Samples were also obtained for prenatal diagnosis of congenital infection; rubella virus RNA was detected in three of seven chorionic villus samples and one of three amniotic fluid samples by RT-PCR, while rubella virus was isolated in only one chorionic villus sample. To demonstrate that the RNA extracted from chorionic villus samples contained amplifiable RNA, a nested RT-PCR was used to detect keratin mRNA. Rubella virus was detected in the placenta in two cases in which the fetus was uninfected, and there was no evidence of rubella virus in the placenta from one case in which the fetus was infected. Thus, detection of rubella virus in chorionic villus samples by RT-PCR may not always correctly predict fetal rubella virus infection. RT-PCR was successfully used for the diagnosis of congenitally acquired rubella in infancy. Rubella virus RNA was detected in cyropreserved or formalin-fixed lens aspirates obtained from infants in India with serologically confirmed congenital rubella but not in samples from controls with inherited cataract.

Rubella is generally a mild disease with few complications. However, when acquired in the first 12 weeks of pregnancy, it usually results in congenital infection associated with one or more severe defects in 80 to 90% of cases. When maternal infection occurs between 13 and 20 weeks, the risk of congenital anomalies declines to about 17%; in some cases, there is serological evidence of infection, but no virus is excreted and no anomalies occur (reviewed in reference 3).

As a result of effective immunization programs, postnatally acquired rubella and congenitally acquired rubella (CAR) have become rare in countries such as the United States and the United Kingdom, although outbreaks of rubella, including cases among pregnant women, have recently occurred in these countries (25, 31, 32). However, in many European countries, which have yet to achieve good vaccination coverage, rubella continues to occur in pregnant women (20). Recent studies suggest that congenitally acquired rubella is responsible for such congenital defects as blindness and deafness in developing countries, and this imposes a considerable burden on scarce health care resources (29).

We have previously described a reverse transcription (RT)-nested PCR assay (RT-PCR) for detection of rubella virus (RV) RNA (7), and in the present study, we evaluated this assay for the prenatal diagnosis of CAR and the diagnosis of CAR in infancy.

Specimens for prenatal diagnosis were obtained from the United Kingdom and Switzerland, and lens aspirates for diagnosis of CAR were obtained from children attending the Aravind Eye Hospital in southern India.

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MATERIALS AND METHODS

Study population and specimens. (i) Products of conception (POC) received after termination of pregnancy. POC and one curettage sample were received from 13 women who acquired serologically confirmed primary rubella before 15 weeks of pregnancy and from 1 woman who acquired rubella about 2 weeks before conception. POC were also received from four women with confirmed or suspected rubella reinfection during pregnancy (Table 1); the diagnosis was made in accordance with previously established criteria (Table 2 [4]). All women had terminations of pregnancy (TOP) between 1988 and 1993. Informed consent was obtained from patients and controls at St Thomas's Hospital, and we understand that the same procedure was followed at other centers. Mixed POC from six women were obtained by suction curettage; in the other cases, a fetus was received and the organs were removed and processed separately (Table 1). Precautions were taken to avoid cross-contamination by washing each organ separately and using separate instruments. The fetus from patient 12 was not available (Table 1), but curettage samples were obtained following a dilatation and curettage. In three cases (patients 4, 7, and 16; Table 1), a small fragment of placenta was taken to simulate a chorionic villus sample (CVS) to determine if the results obtained were concordant with those obtained with a larger sample of placenta. Most specimens were sent by courier from the referring laboratory, frozen, and stored at −70°C for testing at a later date or were sent frozen on dry ice. The serological diagnoses were made by the referring laboratories, which were all in the United Kingdom, but in some cases the referring laboratory requested confirmation of the RV-specific immunoglobulin M (IgM) result in our laboratory by M-antibody capture radioimmunoassay (35). Negative controls, which consisted of placental and fetal tissues and CVS obtained from nine women undergoing TOP between 7 and 12 weeks of gestation for reasons other than rubella, were tested by RT-PCR. Sera obtained from these women were tested for RV-specific IgG and IgM antibodies; none had evidence of recent RV infection.

(ii) Specimens received for prenatal diagnosis. Seven CVS, three samples of amniotic fluid (AF), and two fetal blood samples were obtained from seven women for prenatal diagnosis (Table 3). Samples were frozen and transported to our laboratory on dry ice, but not all were frozen on receipt. Fetal blood samples

TABLE 1. Comparison of RT-PCR and virus isolation for detection of RV in POC obtained after maternal RV infection

Patient group and no.	Sample	Gestational age (wk) at time of:		Result by:	
		Maternal RV infection	TOP	RV RT-PCR	Virus isolation
Primary RV infection					
1	POC	−2	8	−	−
2	POC	4	7	+	+
3	POC	5.5	10	−	+
4	Placenta	7	10	−	−
	CVS			−	−
	Fetal tissue			+	+
5	Placenta	<8	23	NT[a]	−
	Liver biopsy			NT	−
	Heart biopsy			NT	−
6	POC	7.5	12	+	+
7	Placenta	9	12	+	+
	CVS			+	+
	Lung			+	+
	Kidney[b]			+	+
8	POC	10	NK[c]	+	+
9	Ear	11	14	−	−
	Internal organs[d]			−	−
	Heart			−	−
	Brain			−	−
10	Placenta	10–12	23	+	−
	Brain			+	−
	Lung			+	−
	Spleen			+	−
11	Placenta	12	14.5	−	−
	Fetal tissue			−	−
12	Curettage	14	18	−	+
13	Placenta	13.5	15	+	+
	Liver			−	−
	Lung			−	−
	Heart			−	−
	Brain			−	−
14	Liver	NK	NK	−	−
RV reinfection					
15	POC	4	12	−	−
	Fetal tissue			−	−
16	Placenta	5.5	10	−	−
	CVS			−	NT
	Eye			−	−
	Lung			NT	−
	Kidney			−	NT
	Head			−	−
17	Placenta	12	19	+	+
	Eye			NT	+
	Heart			+	+
	Liver			+	NT
	Lung			+	+
18	Brain	13	16–18	−	−
	Heart			−	−
	Kidney			−	−
	Lung			−	−

[a] NT, not tested.
[b] Previously reported as RV negative by RT-PCR (7). RV RNA was detected after retesting of a stored sample.
[c] NK, not known.
[d] Mixture of lung, liver, and spleen.

TABLE 2. History of rubella contact, vaccination, and previous RV antibody screening and serological results used to diagnose RV reinfection

Patient no.	Rubella contact(s)	Yr of vaccination	Time(s) (yr) of previous RV antibody detection	Results in affected pregnancy		Reinfection[b] diagnosis	Outcome of pregnancy (yr)
				Avidity IgG	Specific IgM[a]		
15	Adult, child	None	1988	High	25	Probable	TOP (1989)
16	Own child	1976[c]	1986	NA[d]	Positive[e]	Probable	TOP (1989)
17	Own child	None	1981, 1986	High	17	Confirmed	TOP (1988)
18	Son	1973	1981	NA	8	Probable	IUD[f] (1989)

[a] Results are in arbitrary units; the cutoff is 3.3.
[b] Gestational age at reinfection is shown in Table 1.
[c] Documented.
[d] NA, not available.
[e] RV-specific IgM detected by enzyme immunoassay in referring laboratory.
[f] IUD, intrauterine death.

were tested for RV RNA by RT-PCR and for RV-specific IgM by M-antibody capture radioimmunoassay.

(iii) Lens aspirates. Lens aspirates were obtained from infants 2 to 12 months old attending the Aravind Eye Hospital, Madurai, Tamil Nadu, India. Fifteen lens aspirates were obtained from 12 infants with CAR, serologically confirmed by detection of RV-specific IgM by M-antibody capture radioimmunoassay (Table 4). As controls, eight lens aspirates were obtained from seven infants 4 to 9 months old with autosomal dominant inherited cataract (one parent had cataract) and no other features of congenital rubella and whose mothers had no history of rash during pregnancy. These infants were RV IgM negative and therefore had no serological evidence of congenital rubella.

Lens aspirates were obtained during surgery to remove the cataract. The anterior chamber of the eye was entered through the cornea with a 24-gauge needle, and the anterior capsule of the lens was incised. The lens material was aspirated through an irrigation aspiration cannula into a 2-ml syringe, resulting in a mixture of the lens material, aqueous fluid, and balanced saline solution. In 10 cases, the lens material was transferred to a sterile ampoule, sealed, immediately placed in liquid nitrogen, and later transported to London by air on dry ice. Subsequent samples were put into 5% formal saline (5% formaldehyde, 0.78% NaCl, 0.65% Na_2HPO_4, 0.35% NaH_2PO_4, pH 7.0) as liquid nitrogen was not available. They were stored at room temperature for up to 6 weeks before transport to London and immediate processing on arrival.

Preparation of RNA. (i) POC and prenatal samples. Whenever possible, samples were divided into two and each piece was processed separately. Total RNA was extracted from all samples by using RNAzol-B (Biotex Laboratories, Inc., Houston, Tex.) in accordance with the manufacturer's instructions. All samples were tested in duplicate on one or more occasions to give at least four PCRs per sample.

(ii) Lens aspirates. Total RNA was extracted from frozen lens tissue by using RNAzol-B. Formalin-fixed tissue was centrifuged at 10,500 × *g* for 5 min, the formalin was removed, and the pellet was washed twice in 75% (vol/vol) ethanol and dried briefly under a vacuum before extraction with RNAzol-B. Each specimen was tested in duplicate on one or more occasions.

Detection of RV RNA by RT-PCR. RV RNA was detected by a previously described RT-PCR (7), which has been shown to be specific for RV RNA and sufficiently sensitive to detect 0.1 50% tissue culture infectious dose or approximately two synthetic RNA copies. The oligonucleotides used were located within the E1 open reading frame of the RV genome. The external oligonucleotides R2 (5′ CAA CAC GCC GCA CGG ACA AC 3′) and R7 (5′ CCA CAA GCC GCG AGC AGT CA 3′) produce a 185-bp PCR product, and the internal pair R11 (5′ CTC GAG GTC CAG GTC CYG CC 3′ [Y = C or T]) and R8C (5′ GAA TGG CGT TGG CAA ACC GG 3′) produce a 143-bp product. RT was performed in 20-μl volumes containing 12.5 U of Moloney murine leukemia virus reverse transcriptase (Perkin Elmer, Warrington, United Kingdom), 1 U of Inhibit-ACE RNase inhibitor (5 Prime to 3 Prime Inc., Boulder, Colo.), 3.85 mM primer R7, 1 mM each deoxynucleoside triphosphate (dNTP), 3 mM $MgCl_2$, 50

TABLE 3. Comparison of RT-PCR and virus isolation for RV detection in samples received for prenatal diagnosis from cases of confirmed and suspected primary rubella

Patient no.	Sample(s)	Gestational age (wk) at time of:		Result by:			Outcome of pregnancy
		Maternal rubella	Sampling	RV RT-PCR	Keratin RT-PCR	Virus isolation	
19	CVS	<4[a]	10	−	+	−	Continuing
20	CVS	NK[a,b]	20	−	+	−	Baby normal, no evidence of CAR
21	CVS	3–9	15	+	+	−	TOP, NT[c]
5	CVS	<8	22	+	NT	−	Fetal IgM not detected, TOP[d]
	AF			−	NT	−	
	Fetal blood			−	NT	NT	
10	AF	10–12	22	+	NT	−	TOP[e]
22	CVS	12–13	17	+	NT	+	TOP, NT
23	CVS	13.5	16	−	+	−	
	CVS	13.5	23	−	+	−	Fetal IgM detected, pregnancy continued, CAR
	AF			−	NT	−	
	Fetal blood			−	NT	NT	

[a] Maternal RV infection probably occurred prior to conception.
[b] NK, not known.
[c] NT, not tested.
[d] RV not detected in fetal and liver biopsies by virus isolation (Table 1).
[e] RV detected by RT-PCR in placenta and fetal organs (Table 1).

TABLE 4. Details of patients, RV RT-PCR results obtained with cataractous tissue, and storage of tissue prior to testing

Patient no. (eye)[a]	Age (mo)	Clinical ophthalmic features[b]	Nonocular feature(s)[c]	Maternal symptoms during pregnancy	Infant RV IgM[d]	RV RT-PCR result[e]	Storage method[f] (time [wk])
24	3	Cat, MT, CH	VSD, PDA, deafness	Rash, fever	+	+	LN (5)
25	2	Cat, MT	VSD, deafness	Rash, fever	+	+	LN (6)
26	2	Cat, MT, CH	None	None	+	+	FS (2)
27	3	Cat, MT	VSD	Rash, fever	+	+	FS (2)
28 (R)	9	Cat, MT	None	Rash, fever	+	+	FS (2)
28 (L)	9	Cat, MT	None	Rash, fever	+	+	FS (2)
29 (R)	9	Cat, MT	None	Rash, fever	+	+	FS (2)
29 (L)	9	Cat, MT	None	Rash, fever	+	−	FS (2)
30	12	Cat, MT	VSD, deafness	Rash, fever	+	+	FS (3)
31	5	Cat, MT	None	Rash, fever	+	−	FS (3)
32	6	Cat, MT	None	Rash, fever	+	±	FS (5)
33	8	Cat, MT	None	Rash, fever	+	±	FS (6)
34	2	Cat, MT, CH	VSD	Rash, fever	+	−	FS (6)
35 (R)	7	Cat, MT	VSD	Rash, fever	+	−	FS (6)
35 (L)	7	Cat, MT	VSD	Rash, fever	+	±	FS (6)

[a] R, right; L, left eye.
[b] Cat, cataract; MT, microphthalmos; CH, corneal haze.
[c] VSD, ventricular septal defect; PDA, patent ductus arteriosus.
[d] Serum taken at the same time as lens aspirate.
[e] +, positive; −, negative; ±, equivocal.
[f] LN, liquid nitrogen; FS, 5% formal saline.

mM KCl, 10 mM Tris-HCl (pH 8.3), and a 3-μl RNA sample. The reaction mixture was incubated in a thermal cycler (Omnigene; Hybaid Ltd., Middlesex, United Kingdom) at 37°C for 10 min, 42°C for 30 min, 99°C for 7 min, and 37°C for 2 min. For PCR amplification, 80 μl of PCR mixture was added to each RT reaction to give final concentrations of 2 mM $MgCl_2$, 200 μM each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.77 μM primers R2 and R7, and 2.5 U of *Taq* polymerase (Amplitaq; Perkin Elmer). The reaction mixture was incubated in a thermal cycler (Omnigene) as follows: 95°C for 3 min; 40 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min; and 72°C for 5 min. For nested PCR, 1 μl of the first-round PCR product was added to 99 μl of the nested PCR mixture containing 1.5 mM $MgCl_2$, 200 μM each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.77 μM primers R11 and R8C, and 2.5 U of *Taq* polymerase (Amplitaq). The nested PCR was incubated with the same thermal cycler and temperature parameters as the initial PCR, but the cycle number was reduced from 40 to 25. Ten microliters of the amplified DNA product was resolved by agarose gel electrophoresis on 2% gels and visualized by ethidium bromide staining. Amplification of a 143-bp product in the nested PCR indicated the presence of RV-specific RNA in the RNA sample analyzed. Negative control RNA extractions, sterile water reagent blanks, high and low positive controls, and precautions taken to prevent contamination of PCRs were as previously described (7).

Detection of keratin mRNA by RT-PCR. To demonstrate that the RNA extracted from CVS contained amplifiable RNA, RT-PCR was used to detect mRNA transcripts of the human keratin gene, which is found in many human tissues, including the placenta. The external oligonucleotides KER1 (5′ TGA AGA TCC GTG ACT GGT ACC 3′) and KER2 (5′ ATG TCG GCT GCT TCC ACA CTC AT 3′) produce a 220-bp product (2, 19). The internal oligonucleotides KER3 (5′ GAG GCA GCG GCC TGC TGA G 3′) and KER4 (5′ GGC CAG ACG GGC ATT GTC AA 3′) produce a 133-bp product and were designed from the keratin gene sequence (26). The reagents and conditions for keratin RT and the first-round PCR were the same as those used for RV RT-PCR, except that KER2 was used to prime RT, the PCR annealing temperature was 54°C, and the number of cycles was 30. For nested PCR, 1 μl of the first-round PCR product was added to 49 μl of the nested PCR mixture containing 1 mM $MgCl_2$, 200 μM each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 0.77 μM primers KER3 and KER4, and 1.1 U of *Taq* polymerase (Promega, Southampton, United Kingdom). The nested PCR was incubated in a thermal cycler (Omnigene) as follows: 95°C for 3 min; 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and 72°C for 7 min. The amplified DNA product was visualized as described above.

RV isolation in cell culture. Placental and fetal tissues tested at St Thomas's Hospital were passaged twice in Vero cells, and RV was identified by indirect immunofluorescence in RK13 cells by using sheep anti-RV serum (5). Specimens from patients 19, 20, 21, and 22 (Table 3) were tested in the referring laboratory by using Vero cells and a monoclonal antibody specific for RV hemagglutinin (27). Lens aspirates were not tested by virus isolation.

RESULTS

Detection of RV in placental and fetal tissues received after TOP. (i) Primary infections. Twenty-nine samples from 13 women with confirmed or suspected primary rubella in pregnancy were tested by both RT-PCR and virus isolation, and three samples from patient 5 were tested by virus isolation only. RV RNA was detected in specimens from seven patients (54%) by RT-PCR, and RV was isolated from eight patients (61.5%) (Table 1). Table 5 demonstrates that overall, RV was detected in specimens from 70% (7 of 10) of women who contracted rubella in the first 12 weeks of pregnancy by RT-PCR and/or virus isolation. RV was isolated from specimens from 6 of these 10 women, and RV RNA was detected in specimens from 6 of 9 of these women.

Concordant results were obtained for 23 (79%) of the 29 samples tested by both techniques (Tables 1 and 6); RV was detected in 9 and not detected in 14 samples by both RT-PCR and virus isolation. Of the remaining six samples, RV was detected by RT-PCR and not by virus isolation in the four samples from patient 10 and by virus isolation but not by RT-PCR in one POC and one curettage sample (patients 3 and 12, respectively; Table 1).

(ii) Reinfections. Seventeen samples from the four patients with RV reinfection were tested (Table 1). Of the 12 samples tested by both techniques, 3 from patient 17, who had a confirmed reinfection at 12 weeks of gestation (Table 2), were positive by both RT-PCR and virus isolation. The nine samples from the other three patients were negative by both RT-PCR and virus isolation.

TABLE 5. RV detection by RT-PCR and virus isolation in placental and fetal tissues obtained from 10 women with primary rubella in the first 12 weeks of pregnancy

Sample(s)	No. of women RV positive/no. tested (% positive)		No. of women positive by RT-PCR and/or virus isolation (% positive)
	RT-PCR	Virus isolation	
Placenta	2/4 (50)	1/5 (20)	2/5[a] (40)
Fetal tissues	3/5 (60)	2/6 (33)	3/6[a] (50)
Mixed POC	3/4 (75)	4/4 (100)	4/4 (100)
Total	6/9 (67)	6/10 (60)	7/10 (70)

[a] Samples from one patient were not tested by RT-PCR.

TABLE 6. RV detection by RT-PCR and virus isolation in POC and samples received for prenatal diagnosis and tested by both techniques

Source of samples	No. tested	No. (%) samples RV positive		No. (%) of results concordant
		RT-PCR	Virus isolation	
Primary infections	29	13 (45)	11 (38)	23 (79)
Reinfections	12	3 (25)	3 (25)	12 (100)
Subtotal	41	16 (39)	14 (34)	35 (85)
Prenatal testing	10	4 (40)	1 (10)	7 (70)
Total	51	20 (39)	15 (29)	42 (82)

Overall, of the 41 samples tested by both techniques, 16 (39%) were positive by RT-PCR and 14 (34%) were positive by virus isolation (Table 6). The overall agreement between RT-PCR and virus isolation was 35 (85%) of 41. The three simulated CVS gave the same results as placental samples from the same patients (Tables 1). No RV RNA was detected in two CVS, six placental samples, and four fetal tissue samples obtained from nine patients with no evidence of recent RV infection.

Detection of RV by RT-PCR and virus isolation in specimens received for prenatal diagnosis. Twelve specimens were received from seven women in whom rubella was confirmed or suspected during the first 14 weeks of pregnancy. However, when all information was available, it was concluded retrospectively that two of these patients, 19 and 20, had probably been infected prior to conception; no RV was detected in CVS from these patients. RV RNA was detected in four specimens (three CVS and one AF sample), all obtained from women who had acquired rubella in the first 13 weeks of pregnancy (Table 3), although RV was isolated from only one of these specimens (a CVS from patient 22). RV was not detected by either RT-PCR or virus isolation in the other eight specimens. Five CVS, from patients 19, 20, 21, and 23, in which no RV RNA was detected were tested for keratin mRNA by RT-PCR. The positive keratin mRNA results demonstrated that RNA was present and that it was suitable for detection by RT-PCR (Table 3).

RV RNA was detected in AF from patient 10, in whom there was evidence of fetal infection as RV was detected in fetal organs obtained at TOP (Tables 1 and 3). Although RV was detected by RT-PCR in a CVS from patient 5, no RV was detected in AF and there was no evidence of fetal infection; RV RNA or RV-specific fetal IgM was not detected in fetal blood obtained at 22 weeks, and no RV was detected in liver and heart biopsies by virus isolation. No RV was detected in AF or two CVS from patient 23, although RV-specific IgM was detected in fetal blood obtained at 23 weeks. This patient had rubella at 13.5 weeks, and her infant had serological evidence of intrauterine infection, but there was no evidence of virus excretion, and no congenital anomalies were noted at birth.

Detection of RV by RT-PCR in lens aspirates. RV RNA was detected by RT-PCR in 8 (53.3%) of 15 samples of lens tissue from 7 (58.3%) of the 12 infants with confirmed CAR; positive samples had been stored in liquid nitrogen or formalin for 3 weeks or less (Table 4). RV RNA was detected in five (83%) of six samples stored in formalin for 2 weeks or less but in only one of seven samples stored in formalin for more than 2 weeks. Three samples gave equivocal results in that a positive result was obtained with one of the PCR duplicates on two separate occasions; these three samples had been stored in formalin for 5 to 6 weeks. The four samples which were negative had been fixed in formalin for 2 to 6 weeks. RV RNA was not detected in lens tissue samples from the 11 controls, which had all been stored in liquid nitrogen for 1 to 6 weeks. When lens tissue had been stored in liquid nitrogen, there was insufficient tissue for results to be confirmed by virus isolation.

DISCUSSION

As the clinical diagnosis of rubella is unreliable, rapid laboratory diagnosis of rubella in pregnancy is critical to the decision whether a TOP should be offered (3). The risk of congenital infection may usually be estimated by establishing the gestational age at the time of maternal infection. However, it may be useful to attempt prenatal diagnosis of intrauterine infection under circumstances in which the risk is difficult to establish, such as when there is no history of rash in the mother, maternal serology is inconclusive, infection has occurred between 13 and 20 weeks of gestation, or maternal reinfection is confirmed or suspected in the first trimester (4, 33). It is possible to make an intrauterine diagnosis by detection of RV in such specimens as CVS and AF. DNA amplification techniques, such as PCR, may be regarded as ideal techniques for detection of viral nucleic acids in these specimens as they are very sensitive and rapid (15, 22–24).

Detection of specific DNA in AF, which may be obtained at amniocentesis at about 16 weeks of gestation, has been found to be valuable for intrauterine diagnosis of congenital cytomegalovirus and toxoplasmosis (15, 22, 23, 34). Virus or viral nucleic acids may also be detected in CVS, which may be taken from 10 weeks of gestation onwards (14), or in fetal blood, as for parvovirus B19 (11). Evidence of fetal infection may also be obtained by testing fetal blood, obtained by cordocentesis, for specific IgM (13). Fetal blood is not taken for this purpose until about 22 weeks of gestation, when fetal IgM becomes detectable; however, virus-specific IgM levels may be low at this time and therefore false-negative results may be obtained (18, 22). Obstetricians are aware of these developments and frequently require advice on whether PCR is likely to be of value for determining if fetal infection with RV has occurred, even when serological data and gestational age provide a good assessment of risk.

Although several investigators have reported the development of RV RT-PCR with a view to its use for prenatal diagnosis of CAR (7, 9, 16), there has been only one published preliminary report of its use for this purpose (24). Our RT-PCR has been shown to compare well with virus isolation for detection of RV in pharyngeal swabs and POCs (7). In the larger series of POCs presented here, we obtained evidence of intrauterine infection by RT-PCR in 67% of cases in which primary infection had occurred in the first 12 weeks of pregnancy. When both RT-PCR and virus isolation were used, the rate of infection was 70% (Table 5), which is higher than that reported by Enders (17) but lower than the 81 to 88% reported by others (12, 30, 36). RV was also detected in POCs from one (25%) of four women with confirmed or suspected reinfection in the first 13 weeks of pregnancy. This confirms the results of other studies suggesting that the risk of fetal infection is much less after reinfection (approximately 8%) than after primary infection (4, 33).

Discordant results were obtained for 6 (15%) of 41 specimens tested by both RT-PCR and virus isolation (Table 6). RV was detected by RT-PCR alone in placenta and three fetal organs from patient 10. The fact that RV was detected in two samples (one POC and one curettage sample) by virus isolation but not by RT-PCR may have been due to poor RNA

extractions. Unfortunately, insufficient amounts of these specimens were available for testing by keratin RT-PCR.

When specimens obtained for prenatal diagnosis were tested, results of RT-PCR and virus isolation were in agreement for 7 of the 10 specimens tested by both techniques (Tables 3 and 6). RV was detected in two CVS and one AF sample by RT-PCR alone, which may reflect a greater sensitivity of RT-PCR for testing of very small tissue samples such as CVS. Detection of keratin mRNA by RT-PCR was used to demonstrate that RNA extracted from most CVS was suitable for RT-PCR analysis. Such a control is necessary when very small tissue samples are tested and transport and storage of specimens may not be ideal, to identify possible false-negative results.

Our results demonstrate that RT-PCR is sufficiently sensitive to detect RV in CVS. RV isolation is less suitable, as it is technically demanding and a result may not be obtained for 3 to 4 weeks. However, the results of POC testing by both RT-PCR and virus isolation demonstrate that detection of RV in CVS may not predict fetal infection accurately. Thus, RV may be detected in placenta but not in fetal organs (e.g., patients 13 [Table 1] and 5 [Table 3]). Previous studies have also demonstrated that RV may be detected in the placenta but not in the fetus (1, 12, 36). Thus, an undamaged fetus might be lost if a pregnancy were terminated as a result of testing only a CVS. Conversely, RV may be detected in fetal tissue but not in placenta (e.g., patient 4); this has also been reported by others (12, 36). A false-negative result might therefore be obtained by testing only a CVS. This could be due to a sampling effect if the distribution of virus in the placenta were not uniform. It is also possible that the fetus may be infected but undamaged following maternal infection between 13 and 20 weeks (e.g., patient 23) (30) or following inadvertent immunization during pregnancy (reviewed in reference 3).

RV has been detected in AF from cases of CAR by RT-PCR (6) and virus isolation (1, 10, 34). Unfortunately, we received too few samples of AF to fully evaluate its use for prenatal diagnosis of CAR.

We have shown in this and our previous report (7) that RT-PCR is specific and sufficiently sensitive to detect RV RNA in very small tissue samples; however, our results highlight the limitations of RT-PCR for the intrauterine diagnosis of congenital rubella. Detection of RV RNA in CVS may not always be associated with fetal infection and therefore does not justify the 2.3% risk of spontaneous fetal loss following transabdominal chorionic villus sampling (14). Use of AF may be more desirable because of the lower risk involved with sampling, but further investigation of its efficacy is required. Results of this study should dissuade obstetricians from collecting CVS for prenatal diagnosis of congenital rubella by RT-PCR when good clinical and serological data are available.

Although our studies suggest that detection of RV by RT-PCR is of limited value for prenatal diagnosis, this technique may have a useful role in establishing or confirming the diagnosis of CAR. It is necessary to differentiate congenital cataract due to CAR from cataract due to other causes, as children with CAR are at increased risk of other sight-threatening complications, such as glaucoma, retinal and macular degeneration, and diabetic retinopathy, in later life (21).

Although our RT-PCR was 100% specific for detection of RV in lens tissue, the sensitivity of the assay appeared to be affected by the storage time in formalin. Bresters et al. (8) have shown a loss of hepatitis C virus RNA and albumin mRNA in liver tissue fixed in buffered formalin over a period of 3 weeks. However, it is also possible that there was an insufficient sample amount or RV RNA was not present. Further studies are required to establish the optimal conditions for sample transportation in formalin or other fixatives, such as paraformaldehyde.

We have shown that RV can be detected by RT-PCR in cataractous tissue of infants with serologically confirmed CAR and that positive results may be obtained with formalin-fixed specimens transported to a reference laboratory in a distant country. RT-PCR has the advantage that results may be obtained more rapidly than by virus isolation. Formalin fixation offers a considerable advantage for specimens transported from countries where liquid nitrogen or dry ice is not available. Furthermore, as RV has been isolated from lens material obtained from children up to 3 years of age (28), RT-PCR may be of value in confirming the diagnosis of CAR at an age when a serological diagnosis is no longer possible. The RT-PCR described could be performed in industrialized countries to confirm the diagnosis of CAR in developing parts of the world, thereby providing some assessment of the burden induced by CAR. Indeed, it has recently been shown that 26% of cataract cases referred to the Aravind Eye Hospital over a 9-month period were due to congenital rubella.

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