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Hepatitis C Virus Infection in Infants and Children from Argentina

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Hepatitis C virus (HCV) infection is uncommon in children, and its natural history is still unknown. Our aim was to analyze exposure to HCV in 48 infants and children in Argentina and to evaluate consecutive samples in 26 of them to study the outcome of HCV infection in early stages. HCV viremia, as determined by reverse transcription-PCR (RT-PCR) from the 5′ untranslated region, showed continuously positive, occasionally positive, and negative patterns during follow-up. Restriction fragment length polymorphism was performed on RT-PCR-positive samples to evaluate HCV genotype. Genotype 1 turned out to be predominant, and no patient displayed a genotype shift during the observation period. Perinatal HCV infection was predominantly observed in patients born to mothers coinfected with HCV and human immunodeficiency virus. HCV viral load was detected by means of the AMPLICOR MONITOR, version 2.0, kit. No correlation was observed between HCV viral load and alanine aminotransferase and aspartate aminotransferase levels, although we detected a trend towards higher levels among patients displaying consecutive positive HCV RT-PCR results. Our results demonstrate that pediatric HCV infection is characterized by high viral loads and diverse HCV viremia patterns, independent of both age and route of transmission in the population under study. Further research is necessary to determine whether the high rate of HCV replication is related to virus variability or to host immune response.

Since its discovery in 1989 (7), hepatitis C virus (HCV) has been extensively studied due to its involvement in posttransfusional non-A non-B hepatitis. HCV is rarely diagnosed in the acute phase of infection. Most newly infected subjects show only mild, nonspecific symptoms, if at all, and fulminating hepatitis is rare. The infection has been characterized as an insidiously progressive disease that may result in chronic active hepatitis, cirrhosis, and hepatocellular carcinoma. Chronic HCV is thus considered the main indication for liver transplantation in adults. However, severe complications and death usually occur in adults with cirrhosis, which is estimated to develop in 15 to 20% of those infected (20).

HCV infection in childhood has been studied less, mainly because of its initial asymptomatic course and thus the difficulty in reaching an accurate diagnosis early in life. The main risk factors for HCV transmission to children are maternal HCV infection and transfusion of blood or blood products. This latter issue has nearly disappeared since the introduction of improved blood-screening measures worldwide in 1992. Adolescents who were given a blood transfusion before 1992 are thus still at risk of developing posttransfusional hepatitis and its complications. Hence, perinatally acquired HCV and intravenous drug use are likely to be the only significant sources of pediatric and adolescent HCV infection, respectively (17).

The outcome of infection is extremely variable when acquired in childhood, and its natural history in children is still unknown. Long-term outcome is relevant in pediatric patients, as they may still be young adults after 20 to 40 years of infection. Furthermore, it is not feasible to predict in the early stages which patients have a more somber prognosis (16, 17). Nor is it known whether the risk of chronic disease is higher for patients infected at birth than for those infected at an older age. It has been documented that persistent infection develops in 85% of infected newborns, whereas chronic hepatitis and cirrhosis have been found in 70 and 20%, respectively, of those chronically infected (31). Furthermore, persistent viremia in children has been estimated to range from 45 to 86% of cases (16) and shown to remain relatively stable in one patient over time (4).

Considering the silent evolution of HCV infection in children, periodic screening of the infection has become mandatory to prevent liver complications and to predict which patients will develop a more aggressive disease (3, 16).

Several studies have evaluated the epidemiology of HCV infection in children and/or adults in Argentina (12, 14, 23, 24, 26–28), assessing the presence of HCV RNA in a single sample (1, 23, 26, 28).

The aim of the present study was to analyze exposure to HCV in 48 infants and children in Argentina and to evaluate consecutive samples in 26 of them to study the outcome of HCV infection in its early stages. As far as we have been able to ascertain, this is the first study in our region that evaluates a large pediatric cohort and furthermore contributes to the knowledge of the natural history of the infection by assessing biochemical and molecular parameters during a follow-up period longer than 24 months.

MATERIALS AND METHODS

Patients and samples. This is a retrospective and prospective study on pediatric HCV infection. From June 1998 to October 2002, 160 samples from 48 patients at risk for HCV infection were analyzed. In 9 cases, we also retrospectively evaluated samples collected before 1998 which were properly preserved. Forty-four patients attended the hepatology unit at the Ricardo Gutiérrez Children's Hospital, and 4 patients attended the hepatology units at other general hospitals in Buenos Aires, Argentina.

HCV-infected infants and children were defined by the presence of (i) positive

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TABLE 1. Demographic, clinical, and laboratory features of the studied population

Parameter	Value for:		
	Total population	Group A	Group B
No. of patients	48	22	26
Male (*n*)	28	14	14
Female (*n*)	20	8	12
No. of samples	160	22	138
Age (yr)			
Range		19 mo–16 yr	7 mo–16 yr
Median		9	5
Positive anti-HCV antibodies (no. of patients)	48	22	26
Risk factor for HCV transmission (no. of patients)			
Maternal HCV infection	1	0	1
Maternal HCV-HIV coinfection	16	6	10
Transfusion	17	5	12
Unknown	14	11	3
HIV infection (no. of patients)			
Yes	4	3	1
No	44	19	25

anti-HCV antibodies with a signal-to-cutoff ratio of ≥3.8 at or after 18 months of age (2) and/or (ii) positive HCV RNA on two or more separate occasions.

Patients were classified into two groups, and the demographic, clinical, and laboratory features are summarized in Table 1.

Group A comprised 22 patients older than 18 months from whom we obtained only one blood sample each, whereas group B included 26 patients whose HCV infections were monitored for follow-up.

Samples were obtained at regular intervals during follow-up, which was initially every 3 months. After confirmation of HCV infection, samples were collected at longer intervals (6 to 12 months) to monitor HCV viremia. The follow-up period ranged from 3 to 107 months according to the age of the patient.

Anti-HCV antibodies were determined by third-generation HCV enzyme-linked immunosorbent assay (Ortho Diagnostics Systems, Raritan, N.J.).

Blood samples were obtained by vein puncture and collected in sterile tubes. Samples were centrifuged, and plasma was aliquoted in RNase-free tubes and frozen to −80°C within 3 h.

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined by the International Federation of Clinical Chemistry and Laboratory Medicine method by using an autoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. Normal ALT and AST levels were ≤32 and ≤48 IU/liter, respectively, when testing was done at 37°C.

RNA extraction and RT-PCR of 5′UTR. RNA was extracted from 200 μl of plasma with Trizol reagent (GIBCO BRL, Rockville, Md.) and chloroform. Two microliters of RNA MS2 (Roche Diagnostics) was added to the extraction reagent in each tube as an internal control prior to extraction. RNA was precipitated at −20°C with isopropyl alcohol and centrifuged for 30 min at 4°C. The pellet was washed with 800 μl of 75% ethanol, dried, and resuspended in 40 μl of sterile water. To evaluate the efficiency of extraction, 10 μl of RNA was subjected to agarose gel electrophoresis and ethidium bromide-stained MS2 RNA was observed under UV. Reverse transcription (RT)-nested PCR amplification of the 5′ untranslated region (5′UTR) was performed in a Perkin Elmer GeneAmp PCR System 2400 thermocycler as previously described (14). Briefly, 10 μl of RNA was reverse transcribed and amplified by nested PCR with 5′UTR-specific primers. The primers used were as follows: for the first round, sense primer NF5 (5′-GTG AGG AAC TAC TGT CTT CAC GCA G) and antisense primer NR5 (5′-TGC TCA TGG TGC ACG GTC TAC GAG A) (GIBCO BRL); for the second round, sense primer KF2 (5′-TTC ACG CAG AAA GCG TCT AG) and antisense primer 211 (5′-CAC TCT CGA GCA CCC TAT CAG GCA GT) (GIBCO BRL). The RT-PCR mixture contained 50 mM KCl, 10 mM Tris-HCl (pH 8.4), 1.5 mM $MgCl_2$, 0.16 mM (each) deoxynucleoside triphosphate, 0.4 μM (each) primer, 1 U of *Taq* DNA polymerase (Promega), and 2 U of Moloney murine leukemia virus retrotranscriptase (Promega). The second round was done under the same conditions, except for the Moloney murine leukemia virus retrotranscriptase, and with 5 μl of the first-round PCR product. The PCR protocol consisted of one cycle of RT at 42°C for 15 min, followed by the first PCR round at 95°C for 3 min, 30 cycles of 95°C for 1 min and 62°C for 1 min, and one cycle at 72°C for 7 min. The second round was done under the same conditions as the first round. Twenty microliters of the 251-bp second-round PCR product was observed under UV after electrophoresis on a 2% agarose gel containing ethidium bromide.

Negative results were retested by use of the RT-PCR-based AMPLICOR HCV test kit, version 2.0 (low detection level, 50 IU/ml) (Roche Molecular Systems), according to the manufacturer's instructions.

Restriction fragment length polymorphism analysis of 5′UTR amplicons. Five microliters of the amplicons was digested with restriction enzymes according to the method of Davidson et al. (9) with slight modifications. Briefly, amplicons were incubated separately with *Rsa*I/*Hae*III and *Hin*fI/*Mva*I at 37°C for 4 h, and restriction products were subjected to 15% polyacrylamide gel electrophoresis to evaluate the genotype. To determine the viral subtype, amplicons were digested with *Bst*UI at 60°C for 1 h (genotype 1) or *Scr*FI at 37°C for 4 h (genotypes 2 and 3) and further subjected to 12% polyacrylamide gel electrophoresis. Results were expressed as previously described (14).

Determination of HCV viral load. The HCV viral load was determined from a fresh plasma aliquot by using the RT-PCR-based AMPLICOR HCV MONITOR test, version 2.0 (linear range, 600 to 850,000 IU/ml) (Roche Molecular Systems), according to the manufacturer's instructions.

Avoidance of PCR contamination. To avoid PCR contamination, we used separate airflow cabinets for RNA extraction and RT-PCR. In addition, individual sets of micropipettes and aerosol-resistant tips were used for each procedure. Agarose gel electrophoresis was carried out in a separate room. We strictly followed the recommendations of Kwok and Higuchi (19).

RESULTS

To determine HCV infection in children and infants, we analyzed 160 samples from 48 patients. Table 1 summarizes the features of the studied population. Anti-HCV antibodies were considered positive with a signal-to-cutoff ratio of ≥3.8 in all patients.

HCV viremia was assessed for all samples (Table 2). HCV viremia results were positive for 23 patients, occasionally positive for 8 patients, and negative for 17 patients. In particular, in group B, 15 patients had consecutive positive results and 3 patients had consecutive negative results for HCV viremia, whereas 8 patients had three additional viremia patterns. The first pattern was present in 5 of 8 patients who initially showed positive viremia which then became undetectable in subsequent samples (+/− pattern). The second pattern, observed in 1 of 8 patients, showed initially undetectable viremia that became positive in consecutive samples (−/+ pattern). In the third pattern, present in 2 of 8 patients, viremia fluctuated from positive to undetectable and again to positive during the follow-up period (intermittent pattern).

Table 3 summarizes virus genotypes found in HCV-positive samples. The predominant genotype was 1, with subtypes a/c, which cannot be separately detected by the method used, being the most commonly observed subtypes. However, multiple infections with different HCV genotypes or subtypes were not observed. The same genotype and subtype were detected in

TABLE 2. Distribution of HCV viremia patterns

Patient group	Total no. of patients	No. of patients with RT-PCR HCV result				
		+	+/−	−/+	INT[a]	−
A	22	8	0	0	0	14
B	26	15	5	1	2	3
Total	48	23	5	1	2	17

[a] INT, intermittent viremia (see text).

TABLE 3. Analysis of virus genotypes present in HCV-positive samples

Patient group	Total no. of patients	No. of patients with HCV genotype				
		1	1a/c	1b	Other	ND[a]
A	8	1	2	1	0	4
B	23	1	12	6	1	3
Total	31	2	14	7	1	7

[a] ND, not determined.

consecutive samples from each patient during the follow-up period.

It is widely recognized that maternal HCV-human immunodeficiency virus (HIV) coinfection facilitates vertical HCV transmission. Among 17 perinatally infected children, 16 (94%) were born to HCV-HIV-coinfected mothers.

We also evaluated HCV viral load and both serum transaminase values during follow-up (Fig. 1). Despite the fact that patients were asymptomatic during our study, the levels of ALT and AST in serum were elevated in almost 50% of the cases. We also detected persistent high viremia levels in consecutive samples of children and infants, as determined by HCV viral load. Interestingly, we found higher levels of ALT and AST in patients with higher viremia than in those who had a low or undetectable HCV viral load. Among group B patients, 3 of 26 presented an undetectable viral load, although viremia was detected by RT-PCR, but this apparent discrepancy may be explained by the dissimilar sensitivities of the two methods used.

DISCUSSION

In the present study, we retrospectively and prospectively evaluated a cohort of 48 infants and children in Argentina who presented with diverse risk factors for HCV infection, 22 of whom had been referred to our laboratory only once for HCV determination at the time of this study. As it was not possible to obtain more than one blood sample from these patients, the population studied was separated into two groups.

HCV viremia was evaluated in the 22 pediatric patients with one blood sample each (group A). The evaluation of HCV infection among these patients rendered 14 negative HCV viremia results from 5 children born to HCV-HIV-coinfected women, 2 cases of posttransfusional hepatitis under study, and 7 patients with sporadic anti-HCV positive antibodies (data not shown). HCV RNA-negative samples were retested with the more sensitive AMPLICOR assay, and reproducible results were rendered.

On the other hand, due to an exhaustive analysis of pediatric samples obtained during follow-up, we detected diverse patterns of HCV viremia in 26 patients from group B, ranging from persistently negative through intermittent patterns to persistently positive RT-PCR results. We confirmed fluctuating viremia in 8 patients free of treatment, as also previously described for adults and children (6, 12, 13, 15). Group B patients were thoroughly evaluated to determine the features of HCV infection in children and infants in our country and its evolution over time.

It is well documented that maternal HCV-HIV coinfection facilitates HCV transmission from mother to child. Recently, Yeung et al. (31) performed a meta-analysis of 8 studies on mother-to-child HCV transmission and established a weighted rate of vertical HCV transmission of 19.4% ± 2.0% among HCV-HIV-coinfected women. In our study, the majority of patients who became perinatally HCV infected were born to HCV-HIV-coinfected mothers, indicating that vertical HCV transmission is enhanced by maternal HIV coinfection.

In the present study, vertical HCV infection in children younger than 18 months was diagnosed by means of viremia evaluation in consecutive samples, since maternal anti-HCV antibodies may still be present until this age. However, it should be kept in mind that in perinatally infected patients, HCV RNA may be detected in serum at variable times after infection. For example, Dal Molin et al. detected HCV RNA 1 month after birth in an infant born to an HCV-positive mother (8), whereas Ketzinel-Gilad et al. observed positive HCV viremia as early as 2 days after delivery in other patients (18). Hence, the follow-up period proves to be crucial to reach an accurate diagnosis, mainly for perinatal HCV infection.

The genotype 1 predominance detected in our patients was in agreement with previous reports from our country (14, 23, 26) and from South America (21, 22, 25, 29). Surprisingly, genotype assignment was not feasible for 4 samples, due to a

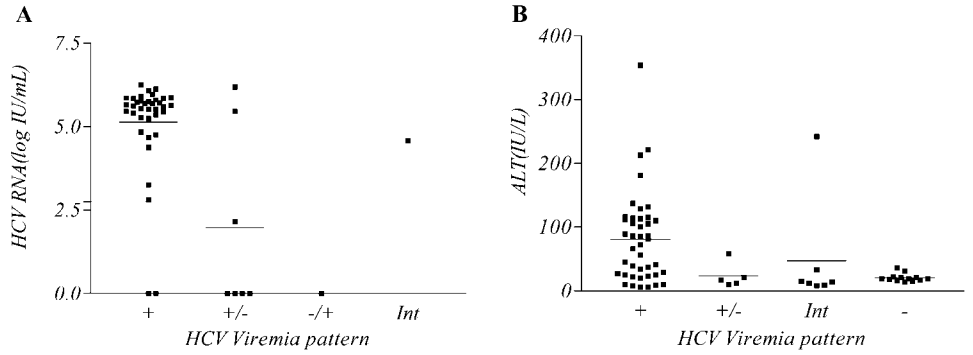

FIG. 1. Analysis of HCV viral load (A) and serum ALT levels (B) in consecutive samples from group B patients for each group of HCV viremia pattern. The short thick black lines represent mean HCV viral load and mean ALT values. The thin line in panel A shows the HCV RNA lower quantitation limit (2.78 log IU/ml). Normal ALT levels were ≤32 IU/liter. Serum AST levels showed a pattern of correlation to HCV viremia similar to the one illustrated for ALT.

DNA restriction pattern with an extra restriction site, distinct from the one described by Davidson et al. (9). Future analysis of the HCV 5′UTR sequence will allow us to define the HCV genotype more accurately in such samples.

A helpful approach for the detection of HCV RNA in the population studied was the evaluation of HCV viral load, which is recommended worldwide for monitoring HCV therapy (16, 20). In this study, we used HCV viral load to evaluate the progression of HCV replication in the host. High levels of HCV viremia persisted in most pediatric patients during follow-up, proving similar to the ones reported by other authors for adult patients (30). These data demonstrate that HCV viral load is unrelated to patient age. Furthermore, we detected a higher mean HCV viral load in patients with continuously positive viremia than in those showing other HCV viremia patterns. Other authors have reported that HCV viral load and liver damage are largely independent in adults (11) and children (4). Thus, we believe that the high HCV viral load observed in our patients can hardly be a predictive factor of liver damage, as it mainly depends on the host's ability to control HCV replication.

The relationship between ALT and HCV viral load in adults and pediatric patients has been widely evaluated. In the present study, normal ALT and AST values were detected irrespective of the HCV viral load displayed. Thus, these biochemical markers of liver function fail to correlate with viral replication (Pearson r^2, 0.07882; two-tailed P value, 0.1647). The absence of serum transaminases and viral load correlation was also reported in other studies of pediatric (4, 5) and adult (10) patients. However, we observed a trend to higher levels of serum transaminases in patients with increased viral loads, but further analysis of a larger cohort will be required for confirmation.

In conclusion, while chronic HCV infection in children and infants is depicted by a high rate of viral replication, the setting up of HCV infection may occur with fluctuating viremia. Thus, the existence of intermittent viremia in children and infants rules out using one blood sample as a diagnostic tool for the assessment of HCV infection and highlights the need for periodic HCV infection monitoring in childhood.

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REFERENCES

1. **Alfonso, V., D. Flichman, S. Sookoian, V. Mbayed, and R. Campos.** 2001. Phylogenetic characterization of genotype 4 hepatitis C virus isolates from Argentina. J. Clin. Microbiol. **39:**1989–1992.
2. **Alter, M. J., W. L. Kuhnert, L. Finelli, and Centers for Disease Control and Prevention.** 2003. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. Morb. Mortal. Wkly. Rep. **52**(RR-3)**:**1–13.
3. **American Academy of Pediatrics. Committee on Infectious Diseases.** 1998. Hepatitis C virus infection. Pediatrics **101:**481–485.
4. **Azzari, C., M. Resti, F. Bortolotti, M. Moriondo, C. Crivellaro, P. Lionetti, and A. Vierucci.** 1999. Serum levels of hepatitis C virus RNA in infants and children with chronic hepatitis C. J. Pediatr. Gastroenterol. Nutr. **29:**314–317.
5. **Casanovas Lax, J., G. Silva Garcia, J. Vargas Romero, M. Nogales Perez, J. Aguayo Maldonado, G. Cruz Guerrero, and J. Gonzalez Hachero.** 1997. Transmisión vertical del virus de la hepatitis C. An. Esp. Pediatr. **47:**627–632.
6. **Chang, M., Y. Ni, L. Hwang, K. Lin, H. Lin, P. Chen, C. Lee, and D. Chen.** 1994. Long term clinical and virologic outcome of primary hepatitis C virus infection in children: a prospective study. Pediatr. Infect. Dis. J. **13:**769–773.
7. **Choo, Q. L., G. Kuo, A. J. Weiner, L. R. Overby, D. Bradley, and M. Houghton.** 1989. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science **244:**359–362.
8. **Dal Molin, G., P. D'Agaro, F. Ansaldi, G. Ciana, C. Fertz, S. Alberico, and C. Campello.** 2002. Mother-to-infant transmission of hepatitis C virus: rate of infection and assessment of viral load and IgM anti-HCV as risk factors. J. Med. Virol. **67:**137–142.
9. **Davidson, F., P. Simmonds, J. Ferguson, L. Jarvis, B. Dow, E. Follet, C. Seed, T. Krusius, C. Lin, G. Medgyesi, H. Kiyokawa, G. Olim, G. Duraisamy, T. Cuypers, A. Saeed, D. Teo, J. Conradie, M. Kew, M. Lin, C. Nuchaprayoon, O. Ndimbie, and P. Yap.** 1995. Survey of major genotypes and subtypes of hepatitis C using RFLP of sequences amplified from the 5′ non-coding region. J. Gen. Virol. **76:**1197–1204.
10. **De Moliner, L., P. Pontisso, G. L. De Salvo, L. Cavalletto, L. Chemello, and A. Alberti.** 1998. Serum and liver HCV RNA levels in patients with chronic hepatitis C: correlation with clinical and histological features. Gut **42:**856–860.
11. **European Paediatric Hepatitis C Virus Infection Network, P. A. Tovo, L. J. Pembrey, and M. L. Newell.** 2000. Persistence rate and progression of vertically acquired hepatitis C infection. J. Infect. Dis. **181:**419–424.
12. **Findor, J., J. Sorda, J. Daruich, E. Bruch Igartua, E. Manero, A. Avagnina, D. Benbassat, J. Rey, and M. Nakatsuno.** 1999. Distribución de los genotipos del virus de la hepatitis C en una población argentina de drogadictos endovenosos. Medicina **59:**49–54.
13. **Freeman, A. J., G. Marinos, R. A. Ffrench, and A. R. Lloyd.** 2001. Immunopathogenesis of hepatitis C virus infection. Immunol. Cell Biol. **79:**515–536.
14. **Gismondi, M. I., M. V. Preciado, I. Badía, A. Ferro, C. Galoppo, and S. Grinstein.** 2001. Estudio y caracterización genotípica de la infección por el virus de hepatitis C en niños. Medicina **61:**815–820.
15. **Granovsky, M., H. Minkoff, B. Tess, D. Waters, A. Hatzakis, D. Devoid, S. Landesman, A. Rubinstein, A. Bisceglie, and J. Goedert.** 1998. Hepatitis C virus infection in the mothers and infants cohort study. Pediatrics **102:**355–359.
16. **Hardikar, W.** 2002. Hepatitis C in childhood. J. Gastroenterol. Hepatol. **17:**476–481.
17. **Jonas, M. M.** 2002. Children with hepatitis C. Hepatology **36:**173–178.
18. **Ketzinel-Gilad, M., S. L. Colodner, R. Hadary, E. Granot, D. Shouval, and E. Galun.** 2002. Transient transmission of hepatitis C virus from mothers to newborns. Eur. J. Clin. Microbiol. Infect. Dis. **19:**267–274.
19. **Kwok, S., and R. Higuchi.** 1997. Avoiding false positives with PCR. Nature **339:**237–238.
20. **Lauer, G. M., and B. D. Walker.** 2001. Hepatitis C virus infection. N. Engl. J. Med. **345:**41–52.
21. **Munoz, G., M. Velasco, V. Thiers, C. Hurtado, J. Brahm, M. Larrondo-Lillo, A. Guglielmetti, G. Smok, C. Brechot, and E. Lamas.** 1998. Prevalence and genotypes of hepatitis C virus in blood donors and in patients with chronic liver disease and hepatocarcinoma in a Chilean population. Rev. Med. Chil. **126:**1035–1042.
22. **Oliveira, M. L., F. I. Bastos, R. R. Sabino, U. Paetzold, E. Schreier, G. Pauli, and C. F. Yoshida.** 1999. Distribution of HCV genotypes among different exposure categories in Brazil. Braz. J. Med. Biol. Res. **32:**279–282.
23. **Oubiña, J., J. Quarleri, M. Rudzinski, C. Parks, I. Badía, and S. González Cappa.** 1995. Genomic characterization of hepatitis C virus isolates from Argentina. J. Med. Virol. **47:**97–104.
24. **Picchio, G. R., M. Nakatsuno, C. Boggiano, R. Sabbe, M. Corti, J. Daruich, R. Perez-Bianco, M. Tezanos-Pinto, R. Kokka, J. Wilber, and D. Mosier.** 1997. Hepatitis C (HCV) genotype and viral titer distribution among Argentinean hemophilic patients in the presence or absence of human immunodeficiency virus (HIV) co-infection. J. Med. Virol. **52:**219–225.
25. **Pujol, F. H., C. L. Loureiro, M. Devesa, L. Blitz, K. Parra, S. Beker, and F. Liprandi.** 2002. Determination of genotypes of hepatitis C virus in Venezuela by restriction fragment length polymorphism. J. Clin. Microbiol. **35:**1870–1872.
26. **Quarleri, J., I. Badía, V. Mathet, and J. Oubiña.** 1999. Epidemiología molecular del virus de la hepatitis C (HCV) en pacientes hemofílicos de edad pediátrica. Prensa Med. Argent. **86:**181–185.
27. **Quarleri, J., B. Robertson, V. Mathet, M. Feld, L. Espínola, M. Requeijo, O. Mandó, G. Carballal, and J. Oubiña.** 2000. Genomic and phylogenetic analysis of hepatitis C virus isolates from Argentine patients: a six-year retrospective study. J. Clin. Microbiol. **38:**4560–4568.
28. **Quarleri, J., B. Robertson, V. Mathet, S. Sinha, I. Badía, B. Frider, A. Ferro, C. Galoppo, S. Sookoian, G. Castaño, and J. Oubiña.** 1998. Genomic and phylogenetic analysis of hepatitis C virus strains from Argentina. Medicina **58:**153–159.
29. **Sánchez, J. L., M. H. Sjogren, J. D. Callahan, D. M. Watts, C. Lucas, M. Abdel-Hamid, N. T. Constantine, K. C. Hyams, S. Hinostroza, R. Figueroa-Barrios, and J. C. Cuthie.** 2000. Hepatitis C in Peru: risk factors for infection, potential iatrogenic transmission, and genotype distribution. Am. J. Trop. Med. Hyg. **63:**242–248.
30. **Sasaki, N., A. Matsui, M. Momoi, F. Tsuda, and H. Okamoto.** 1997. Loss of circulating hepatitis C virus in children who developed a persistent carrier state after mother to baby transmission. Pediatr. Res. **42:**263–267.
31. **Yeung, L. T. F., S. M. King, and E. A. Roberts.** 2001. Mother-to-infant transmission of hepatitis C virus. Hepatology **34:**223–229.