

# TT Virus Infection in Children and Adults who Visited a General Hospital in the South of Brazil for Routine Procedure

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*TT virus (TTV) is a newly described nonenveloped human virus, with a circular, negative-stranded DNA genome, that was first identified in the blood of a patient with posttransfusion hepatitis of unknown etiology. PCR primers and conditions used for TTV DNA amplification may greatly influence the level of TTV detection in serum. Three PCR assays, with different regions of the genome as targets, were used to test TTV DNA in 130 sera from children and adults visiting a hospital in the south of Brazil, most of them for routine procedure. Forty-four percent of adult sera and 73% of sera from children aged 0-10 years were TTV positive with at least one PCR assay. However, the three assays were able to detect only 33%, 35%, and 70% of the total positive samples. Our results showed a high prevalence of TTV infection in the south of Brazil, particularly among young children, and confirmed the necessity of performing several PCR assays to assess the true TTV prevalence in a determined population.*

Key words: liver disease - novel virus - polymerase chain reaction - prevalence - TTV DNA

TT virus (TTV) is a nonenveloped virus (Okamoto et al. 1998) with a circular, negative-stranded DNA genome of 3,818 - 3,853 nucleotides (Erker et al. 1999, Miyata et al. 1999, Mushahwar et al. 1999) that was first identified in 1997 in the blood of a Japanese patient (initials, TT) with posttransfusion hepatitis of unknown etiology (Nishizawa et al. 1997). TTV has been recently shown to infect liver cells (Rodriguez-Iñigo et al. 2000) and to replicate in the liver (Okamoto et al. 2000). However, the association between TTV viremia and liver disease remains uncertain.

The presence of TTV in serum and other body fluids, such as saliva and semen as well as in stool samples, is usually detected by PCR amplification of viral DNA. Very high seroprevalences (30-93%) of TTV have been found in normal healthy populations from different parts of the world, including developed and developing countries (Prescott & Simmonds 1998, Takahashi et al. 1998, Tanaka et al. 1998, Leary et al. 1999, Niel et al. 1999, Okamoto et al. 1999, Handa et al. 2000). In several other studies, however, the true TTV prevalence has been greatly underestimated due to the

use of PCR primers and/or protocols that did not allow the successful amplification of DNAs from all the isolates. Indeed, TTV is a virus with a great genetic heterogeneity. The existence of at least 16 genotypes (1 to 16) has been demonstrated by Okamoto et al. (1999) and a recent study has proposed the classification of TTV into five different virus species (TTV I to V), due to the considerable evolutionary distance separating the strains (Khudyakov et al. 2000). Depending on the PCR assay used, the different TTV genotypes (or species) may or may not be detected.

In this report, we compare three different PCR assays to determine the seroprevalence of TTV in children and adults visiting a hospital in the south of Brazil.

## PATIENTS AND METHODS

From October 1998 to March 1999, blood samples were collected from 130 unselected ambulatory patients (54 male, 76 female, aged 2 to 82 years; mean,  $32 \pm 21$  years) who visited the Federal University Hospital of Florianópolis, State of Santa Catarina, south of Brazil. One hundred and ten (85%) of them went to the hospital for routine procedure; the others were diagnosed with anemia, obesity, urticary, bronchiolitis, astenia, or asthma at sampling time.

Viral DNA was extracted using phenol/chloroform after treatment of 250  $\mu$ l of serum with 0.5 mg/ml of proteinase K in the presence of 0.2 M NaCl, 0.25% SDS, for 4 h at 37°C. After precipitation with ethanol, the pellet was dried and resus-

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pended in 50 ml of distilled water. Three published PCR assays (Takahashi et al. 1998, Tanaka et al. 1998, Leary et al. 1999) were employed to amplify TTV DNA. Tanaka's system is a hemi-nested PCR assay using NG059/NG061/NG063 primers (Okamoto et al. 1998). In this system, the PCR product is a DNA fragment of 271 bp localized in a region of the large open reading frame (ORF1) that has been named N22 region, after the original TTV N22 clone (Nishizawa et al. 1997). Takahashi's system is a single round PCR assay of 55 cycles using the primers T801 and T935 as well as the Ampli-Taq Gold polymerase (Perkin Elmer Applied Systems, Foster City, CA), an enzyme activated after a 9-min incubation at 95°C. Using Takahashi's system, the expected size of the PCR product is 199 bp, and the DNA target lies in the non coding region of the genome, near the 5' end of ORF2. Finally, Leary's system for TTV DNA detection is a nested PCR assay using a set of four primers called set B (Leary et al. 1999). The PCR product is a 223 bp fragment localized in the non coding region, near the 3' end of ORF3.

PCR products (10 µl) were loaded on a 2% agarose gel, electrophoresed, and stained with ethidium bromide to visualize DNA bands.

## RESULTS

Sera from 130 patients (including 91 adults and 39 children aged 0-17 years) who visited the Federal University Hospital, were tested for the presence of TTV DNA by using three established PCR detection assays (Takahashi et al. 1998, Tanaka et

al. 1998, Leary et al. 1999). The results are shown in Table I. Very few serum samples (3 from adults and 2 from children) were positive in all three assays. Thirty-two samples tested positive with either two assays, the Tanaka's assay only, or the Leary's assay only. In addition, 26 sera, including 21 from adults, were positive with the Takahashi's assay only. In all, TTV DNA was detected in 44 samples with the Takahashi's assay, compared to 22 with the Tanaka's assay and 21 with the Leary's assay. Considering that 63 samples were positive in any assay, it could be observed that the Takahashi's assay, although the most adequate to detect TTV in our samples, could amplify DNAs from only 70% of the total TTV positive samples. Sixty-seven (52%) samples remained negative whatever the PCR detection system employed (Table I).

Table II shows the TTV seroprevalences of subgroups of patients when these were divided by gender, age, and living area (urban or rural). There were no significant differences between the subjects with and without TTV DNA in relation to sex ratio and living area. When the subjects were classified according to their age range, the highest rate of TTV infected subjects (73%) was found among the young children (0-10 years).

## DISCUSSION

The use of different PCR primers and conditions in the determination of TTV prevalence has led to discrepant results. For example, Takahashi et al. (1998), using T801/T935 primers, found a

TABLE I  
Detection of TTV DNA in sera of children and adults by using different PCR assays

PCR detection systems			Number (%) of positive samples		
Tanaka	Leary	Takahashi	Adults	Children (0-17 y)	Total
+	+	+	3 (3%)	2 (5%)	5 (4%)
+	+	-	1 (1%)	0 (0%)	1 (1%)
+	-	+	2 (2%)	3 (8%)	5 (4%)
-	+	+	3 (3%)	5 (13%)	8 (6%)
+	-	-	6 (7%)	5 (13%)	11 (8%)
-	+	-	4 (4%)	3 (8%)	7 (5%)
-	-	+	21 (23%)	5 (13%)	26 (20%)
Total of TTV positive with					
Tanaka's assay			12 (13%)	10 (26%)	22 (17%)
Leary's assay			11 (12%)	10 (26%)	21 (16%)
Takahashi's assay			29 (32%)	15 (38%)	44 (34%)
Positive in any assay			40 (44%)	23 (59%)	63 (48%)
Negative in all three assays			51 (56%)	16 (41%)	67 (52%)
Total			91 (100%)	39 (100%)	130 (100%)

TABLE II  
TTV seroprevalence among individuals, with and without symptoms of illness, who visited a general hospital

Patient features	<i>n</i>	TTV DNA Positive (%)
Gender		
Male	54	59
Female	76	42
Age (years)		
0-10	30	73
11-20	13	38
21-30	25	44
31-40	20	30
41-50	18	39
> 50	25	56
Living area		
Rural	69	50
Urban	61	46
Total	130	48

prevalence of 92% in Japanese blood donors, compared to the 12% previously found with the use of NG059/NG061/NG063 primers (Okamoto et al. 1998). Among US blood donors, prevalences of 1-8%, 34%, and 42% have been reported, using the NG059/NG061/NG063 (Charlton et al. 1998, Handa et al. 2000), BF1/BR1/BF2/BR2 (set 2) (Leary et al. 1999), and T801/T935 (Handa et al. 2000) sets of primers, respectively.

The present study was initiated to assess the prevalence of TTV infection in a population visiting a general hospital in the south of Brazil. The Takahashi's assay (T801/T935 primers) appeared to be the most adequate for the detection of TTV DNA in our sera since it was able to efficiently amplify DNAs from 44 virus strains, compared to 21-22 isolates for the two other assays. However, this does not necessarily mean that Takahashi's assay is the most sensitive one, but more likely that it is able to amplify DNAs from genetically distant TTV strains. Indeed, in a previous work performed with serum samples from Rio de Janeiro, Brazil, we demonstrated the capability of Takahashi's assay to detect a large number of genetically distant TTV isolates and showed that the assay was useful to identify mixed infections (Niel et al. 2000). Despite its large spectrum, the Takahashi's assay was able to detect only 44/63 (70%) of the total TTV positive samples (Table I). Our experiments therefore confirmed the necessity of performing several distinct PCR assays to assess the TTV prevalence in a given population, as previously

suggested (Leary et al. 1999, Biagini et al. 2000), at least until a 'universal' PCR assay is described.

A large majority of the sera analyzed here was from people who visited a general hospital for routine procedure. This group was quite representative of the population of the south of Brazil. Taken together with previous studies showing high prevalences (24-65%) of TTV infection in blood donors (Niel et al. 1999), parturient women (Saback et al. 1999), and health care workers (Niel et al. 2000) from Rio de Janeiro, as well as hepatitis patients from São Paulo and Pará states (Pinho et al. 1998), the results presented here constitute additional evidence that a large part of the Brazilian population is chronically infected with TTV.

By calculation of the frequency of TTV viremia by age, a particularly high prevalence (73%) was observed in children 0-10 years old. This corroborated observations made in Japan that showed a high prevalence in a preschool group, followed by a decline during the adolescence and a new elevation among young adults (Yamada-Osaki et al. 1998). However, a similar curve of age-specific TTV seroprevalence was not observed among healthy individuals living in the city of Rio de Janeiro, where the prevalence, measured using the Tanaka's PCR detection system, increased continuously with age (Saback et al. 1999). The reasons which could explain this discrepancy are still not clear. A possible explanation might be that the distributions of the TTV genotypes circulating in Rio de Janeiro and in the south of Brazil are not identical. In this case, it would be interesting to determine the nucleotide sequences of a number of PCR products obtained in this study and compare them to those derived from TTV isolates circulating in other regions of Brazil.

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