

## Laboratory and Epidemiology Communications

### Detection of TT Virus DNA in Human Bile Juice

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Recently, using representational difference analysis, the genome from a novel DNA virus, termed the TT virus (TTV), was isolated from the serum of patients with post-transfusion non-A-G hepatitis (1,2). Due to the genome structure and its banding in buoyant density gradient centrifugation, TTV might be most related to *Circoviridae* viruses among the known animal virus families (3-5). TTV sequence has been detected in sera and liver tissues from liver disease patients, suggesting that TTV would be responsible for some acute and chronic liver diseases of unknown etiology (2,6). It has been reported that TTV infection does not induce significant liver damage (7). We recently reported that TTV infection is widespread in the general population worldwide and suggested that TTV may be a common DNA virus in humans (8). This implies that the routes of TTV transmission may differ from the routes of hepatitis B virus, hepatitis C virus, and hepatitis G virus transmission. The presence of the virus in body fluids other than serum, such as saliva and semen, may affect the routes of viral transmission (9). Accordingly, we used PCR assay to analyze whether TTV is present in bile juice.

Bile juices were obtained from 16 patients who had undergone operation for resection of the gall bladder. The bile juices were collected directly from the removed gall bladders and stored at -80°C until use. Informed consent was obtained from the participants in this study. DNA was extracted from 100 µl of the bile juice, using a nucleic acid extraction kit (SepaGene RV-R, Sanko Junyaku Co., Ltd., Tokyo). The bile juice was diluted two times with phosphate-buffered saline and used for DNA extraction. TTV DNA was amplified by PCR using a method described previously (8). In brief, the thermocycler was programmed first to preheat to 95°C for 10 min in order to activate AmpliTaq Gold DNA polymerase (Perkin-Elmer, Norwalk, Conn., USA) then followed by 55 cycles consisting of 94°C for 20 sec, 60°C for 20 sec, and 72°C for 30 sec using a Perkin Elmer 9700 Thermal Cycler (Perkin Elmer). The sequence of the TTV specific primers were 5'-GCTACG TCACTAACCACGTG-3' (T801, sense primer, nucleotide [nt] 6-25) and 5'-CTBCGGTGTGTAAGTCACTACC-3' (T935, antisense primer, nt 185-204, B=G or C or T) as designed by Takahashi et al. (10) in the 5' end region of TA278 isolate. The PCR products were detected by electrophoresis on 2% agarose gels, stained with ethidium bromide, and photographed under UV light. The sizes of PCR products were

estimated according to the migration pattern of a 50-bp DNA ladder (Pharmacia Biotech, Uppsala, Sweden). All PCR assay were performed in duplicate to confirm specificity.

TTV DNA was detected in the bile juice of 8 (50%) of 16 subjects. To verify that the products amplified by PCR were of TTV origin, sequence analyses of 8 cases were performed using the direct sequencing method. The results revealed that although the nucleotide sequence of TTV DNA from the bile juices had sequence variation among them, all had a high similarity to the TA278 isolate which is the prototype of TTV.

Our results indicate that TTV DNA, besides being present in the blood, can also be found frequently in bile juice. Similar findings were reported by others (11). It is known that the TTV viremia is widespread in the general population worldwide, since the prevalence among patients including healthy populations was found to be over 70% (8). Such an extremely high prevalence of TTV infection in the general population suggests that TTV may be transmissible not only via blood, but also by a nonparental route. Indeed, Okamoto et al. reported that TTV was excreted into the feces, thereby suggesting that TTV would be transmitted not only parenterally, but also nonparenterally by a fecal-oral route (12,13).

In conclusion, TTV DNA was detected frequently in bile juice, suggesting a fecal-oral TTV transmission route.

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