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# TT Virus as a Human Pathogen: Significance and Problems

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**Abstract.** In 1997 TTV was detected using representational difference analysis (RDA) in serum of a patient with posttransfusion hepatitis unrelated to known hepatitis viruses. The genome of TTV is a circular single-stranded DNA molecule of 3852 nt with negative polarity. TTV possibly can be grouped either into the existing family *Circoviridae* or into a recently established virus family ''*Circinoviridae*''. Analysis of the complete DNA nucleotide sequence of TTV identified three partially overlapping open reading frames (ORFs). Neither DNA nucleotide nor corresponding amino acid sequences of TTV do show significant homologies to known sequences. TTV DNA nucleotide sequences amplified by PCR from sera of different patients show considerable sequence variations. Although the natural route of transmission of TTV is still unknown, there is clear evidence for a transmission of TTV through blood and blood products. TTV DNA can be detected in the feces of infected individuals suggesting that it may be possible to attract TTV infection from environmental sources. Since the discovery of TTV, numerous studies have investigated the prevalence of TTV infections in different human population groups all over the world. All these studies are based on PCR detection systems, but the technical aspects of the PCR systems vary significantly between the different investigators. The results of the epidemiological studies do not show a clear picture. The discovery of TTV as a viral agent and particularly the identification of a high percentage of infected carriers in the healthy human population raises the following questions: Firstly, what is the origin and molecular relatedness of TT virus. Secondly, what is the significance of TTV as a human pathogen. And thirdly, what are the exact molecular mechanisms of viral replication. To answer these questions it will be necessary to determine the primary structure and the coding capacity of several TTV patient isolates.

**Key words:** TTV, TT virus, review, PCR technology, *Circoviridae*

## History and Biophysical Properties of TT Virus (TTV)

In 1997 Nishizawa and colleagues used representational difference analysis (RDA), a modified polymerase chain reaction (PCR), to identify a possible infectious agent in serum of a patient with posttransfusion hepatitis unrelated to known hepatitis viruses (1). The investigators succeeded in isolating an amplified DNA fragment comprising 500 nucleotides (nt) of unknown origin (clone N22). This DNA fragment was detected by PCR in fractions of plasma collected from a sucrose density gradient but was not detectable in human genomic DNA. The DNA in the plasma fractions was resistant to DNAse I digestion. These results indicated a possible viral origin of the N22 clone (1). Consequently, primer extension of the original N22 clone was used to identify 3739 nt of the novel viral genome (GenBank accession number AB008394). The virus was termed TT Virus (TTV) according to the initials of the first patient (T.T.).[1] TTV was found to contain a single-stranded DNA

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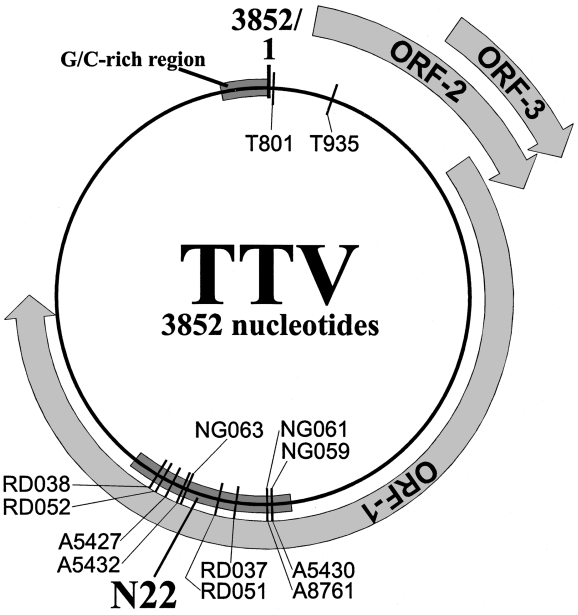


*Fig. 1.* Physical map of the genome of TT virus according to the complete TTV genome sequence available under the GenBank number AF122913. The first clone (N22) (1), the newly described GC-rich region (4,5), three open reading frames (4) and the binding sites of several primer sets described in Table 1 are indicated.

genome and its unenveloped virion has a buoyant density of 1.31 – 1.32 g/cm$^3$ in CsCl (2). However, until now TTV has not been visualized by electron microscopy. So far no cell culture systems have been reported that allow the replication of the virus.

The genome of TTV was first reported to be linear single-stranded DNA (2), a feature common to the genomes of the *Parvoviridae*. However, recent work by two independent groups showed that the viral genome is a circular single-stranded DNA molecule of 3852 nt (3,4) with negative polarity (4) (GenBank accession number AF122913).[2] These investigators proposed to either group TTV into the existing family *Circoviridae* (3) or to establish a new family *Circinoviridae* (4).

The classified members of the family *Circoviridae* are animal viruses with a circular single-stranded DNA genome and include chicken anemia virus (CAV), beak and feather disease virus (BFDV) and porcine circovirus (PCV). The virions of these viruses have an icosahedral structure, no envelope and a buoyant density in CsCl of 1.33–1.37 g/cm$^3$ (5). The genomic DNA nucleotide sequences of these viruses have a size of 2298 nt (6), 1993 nt (7), and 1759 nt (8), respectively.[3] Several plant viruses are also considered as possible members of the family. However, CAV is different from the other circoviruses in electron microscopic appearance, primary structure, and also when their transcription and replication mechanisms are compared (6,7,9–13).

Analysis of the complete DNA nucleotide sequence of TTV identified three partially overlapping open reading frames (ORFs) that resemble the ORFs of chicken anemia virus (CAV) in relative size and location (3) (Fig. 1). The DNA nucleotide and corresponding amino acid sequences of TTV do not show significant homologies to known sequences with exception of 36 nt (genome position 3816–3851; AF122913) in the recently identified GC-rich region of 113 nt that has a 80.6% DNA nucleotide sequence homology to a corresponding sequence in CAV (3). TTV ORF-1 and CAV ORF-L, encoding the structural protein of CAV, are both proteins with an arginine rich domain at the N-terminus (14).

On the basis of these obvious similarities in genome organization, a new genus within in the family Circoviridae, comprising TTV and CAV as new members should be considered. TTV DNA nucleotide sequences amplified by PCR from sera of different patients show considerable sequence variations. However, partial sequences of both TTV ORF 1 and 2 obtained from TTV infected hemodialysis patients remained completely unchanged for timeperiods of up to two and a half years (15). TTV DNA sequences and deduced amino acid sequences of patients (from Heidelberg, Germany), are shown as an example in Fig. 2. This data is in complete agreement with the TTV data published so far. The genetic heterogeneity of TTV has been investigated using genetic distance methods, tree construction and bootstrap verification (2,4,15–17). The investigators suggest a genomic grouping of TTV in two (2,16), three (4,15), or six (17) genotypes. However, the suggested grouping of TTV is preliminary and requires additional genomic studies based on a sufficient number of full-length DNA nucleotide sequences of TTV isolates. Until now, no correlation between TTV genotypes and geographical distribution could be detected (4,16,17).

## Molecular Epidemiology

Since the discovery of TTV, numerous studies have investigated the prevalence of TTV infections in

# A

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N22   1 CTAAGCAAAAAAAACATGAACTATGACAAACTACAAAGTAAATGCTTAATATCAGACCTACCTCTATGGGCAGCAGCATA  80
P01   1 C.TACT..AAAAGACATGAAA..TGAC...GTGCAA..C..A..CC.AG..GCAG.CC.A..AC.G.....AG.ATCA..  80
P02   1 C.TAGT..AAAAGCCATGGAA..TGAC...GTGCAA..C..G..CC.AG..GCAG.CC.A..AC.G.....AG.AGCA..  80
P03   1 C.TAGT..AAATAACATGAAC..TGAC...GTGCAA..C..G..CC.AG..GCAG.CC.A..AC.A.....AG.AGCA..  80
P04   1 C.AACC..AAAAAATATGAAT..TGAC...GTACAA..C..A..CC.AA..TCAG.CT.A..AC.G.....AG.AGCA..  80
P05   1 C.AACC..GAAAAATATGAAT..TGAC...GTACAA..C..A..CC.AA..TCAG.CT.A..AC.G.....AG.AGCA..  80
P06   1 C.AACC..AGATGACTCACAA..TTCA...ACGCAG..C..A..TC.CA..GAGA.CT.G..TC.G.....CT.AGTA..  80
P07   1 C.AACT..AGATGACTCAGCC..CTCA...ACAAGT..C..G..CC.CA..GAGA.CT.A..CC.G.....CT.AGTA..  80
P08   1 C.AACT..AGATACCTCAGTA..TGAC...ACACAG..T..A..TC.CA..CAAG.CA.G..CC.G.....CT.CGTG..  80
P09   1 C.AACT..AGATACCTCAGTA..TGAC...ACACAG..T..A..TC.CA..CAAG.CA.G..CC.G.....CT.CGTG..  80
P10   1 G.ATCA..GACAGACTCAATC..TGAC...GCACGC..C..A..TT.AA..GAAA.AT.A..CA.G.....CG.GGTA..  80
          *++  **++ ++  + +   ** +  ***  + + ** **+**  * +**  ++*  *+**  *+***** +* + +**

N22  81 TGGATATGTAGAATTTTGTGCAAAAAGTACAGGAGACCAAAACATACACATGAATGCCAGGCTACTAATAAGAAGTCCCT 160
P01  81 T..T.ATTTA..A.TC..CTCT..AAGC....G...CACA......C.CATG..TGCC..ACTAC.A..A...AGT..C. 160
P02  81 T..G.ATTTA..A.TC..CTCT..AAGC....G...CACA......C.TATG..TGCC..ACTGC.A..A...AGT..C. 160
P03  81 T..G.ATTTA..A.TC..CTCT..AAGC....G...CACA......C.CATG..TGCC..ACTGC.A..A...AGT..C. 160
P04  81 C..C.ATTTA..A.TC..TGCA..AAGC....G...CCAA......C.CATG..TGCC..ACTAT.A..A...AGT..C. 160
P05  81 C..C.ATTTA..A.TC..TGCA..AAGC....G...CCAA......C.CATG..TGCC..ACTAT.A..A...AGT..C. 160
P06  81 C..A.ACACA..A.AC..CAGC..AGTA....G...TTCA......G.ACAC..CTGC..ATGTG.T..C...AGC..C. 160
P07  81 C..A.ACAAA..G.AC..CAGC..AGTA....G...TACA......G.ACAT..CTGC..ATGTG.T..C...AGC..C. 160
P08  81 C..A.TCTCC..A.AC..CAGC..AGTA....C...CACA......G.GCAC..CTGT..ATGTG.T..T...AGC..C. 160
P09  81 C..A.TCTCC..A.AC..CAGC..AGTA....G...CACG......G.GCAC..CTGT..ATGTG.T..T...AGC..C. 160
P10  81 T..C.ACGCA..A.AC..TGCC..GAGC....C...CTCT......G.CATG..CGCC..AGTAG.T..G...TGT..A. 160
          ** *+ +  **+*+ **   +**+++ **** ***   +****** *  + ** +  **+ ++ *+** ***+* ** *

N22 161 TTACAGACCCACAACTACTAGTACACA--CAGACCCCACAAAAGGCTTTGTTCCTTACTCTGTA 222
P01 161 TT..A.AC..C..AC.AA...TA....--..GA...CACT.AA..C.TT..A..T..TTCTT.A 222
P02 161 TT..A.AC..C..GC.AA...TA....--..AA...CAAT.AA..C.TT..A..T..TTCTT.A 222
P03 161 TT..A.AC..C..GC.AA...TA....--..AA...CAAC.AA..C.TT..A..T..TTCTT.A 222
P04 161 TT..A.AC..C..AC.GC...TA....--..AA...AAAT.AA..C.TT..T..T..CTCCC.A 222
P05 161 TT..A.AC..C..AC.GC...TA....--..AA...AAAT.AA..C.TT..T..T..CTCCC.A 222
P06 161 AC..A.TA..A..AC.GT...AC....A-..AT...CTC-.GA..A.AC..A..C..CAGCT.T 222
P07 161 AC..A.TA..A..AC.AT...AC....A-..AT...CTC-.GA..T.AC..G..T..TAGCC.C 222
P08 161 AC..A.TG..A..AC.AT...AT....A-..AC...CTC-.GG..A.AC..G..C..CAGTT.T 222
P09 161 AC..A.TG..A..AC.AT...AT....A-..AC...CTC-.GG..A.AC..G..C..CAGTT.T 222
P10 161 AC..T.TT..C..AA.GA...A-....AG..AT...CTC-.GG..C.TT..A..A..CAGCT.T 222
          + **+*+ ** **+ *  ***+ ****  **+ *** +  *++** *+ **+** ** +   *
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# B

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N22  1 LSKKNMNYDKLQSKCLISDLPLWAAAYGYVEFCAKSTGDQNIHMNARLLIRSPFTDPQLLVHTDPTKGFVPYSV 74
P01  1 LT.KNMN.D.VQ....ISDL.L..AA..YL.F.A.S.G.Q..HM.A.LLI.S.F.D..LLVHTN.NK.F....L 74
P02  1 LT.KNMN.D.VQ....ISDL.L..AA..YL.F.A.S.G.Q..HM.A.LLI.S.F.D..LLVHTN.NK.F....L 74
P03  1 LS.NNMN.D.VQ....VADL.L..AA..YL.F.S.S.G.T..HM.A.LLI.S.F.D..LIVHTN.NK.F....L 74
P04  1 LS.KAME.D.VQ....VADL.L..AA..YL.F.S.S.G.T..HM.A.LLI.S.F.D..LIVHTN.NK.F....L 74
P05  1 LT.KDMK.D.VQ....VADL.L..AS..YL.F.S.S.G.T..HM.A.LLI.S.F.D..LIVHTD.TK.F....L 74
P06  1 LT.DDSQ.S.TQ....IENL.L..SV..YT.Y.S.V.G.S..EH.C.CVI.S.Y.V..LLDHNN.LR.Y....F 74
P07  1 LT.DDSA.S.TS....IENL.L..SV..YK.Y.S.V.G.T..EH.C.CVI.S.Y.V..LLDHNN.LR.Y....L 74
P08  1 LT.DTSV.D.TQ....IQDM.L..SV..FS.Y.S.V.A.T..EH.C.CVI.S.Y.V..LLDHNN.LR.Y....F 74
P09  1 LT.DTSV.D.TQ....IQDM.L..SV..FS.Y.S.V.G.T..EH.C.CVI.S.Y.V..LLDHNN.LR.Y....F 74
P10  1 VS.TDSI.D.AR....IEKL.M..AV..YA.Y.A.S.A.S..DM.A.VVM.C.Y.V..MIDTSN.LR.F....F 74
         ++*++  *+*  ****+ ++*+**+ **+ *+*+* *+* **+ * * ++*+*+* **++  +** +*+****
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*Fig. 2.* Multiple alignment of the DNA nucleotide sequences (A) and deduced amino acid sequences (B) of PCR products obtained from several individuals in Heidelberg, Germany. Below the aligned sequences, well-conserved and identical nucleotide or amino acid positions are indicated by plus and asterisk symbols, respectively. Dashes indicate gaps to achieve maximal nucleotide matching. N22, sequence of the first clone N22 (1), P01 to P10, patients 1–10.

*Table 1.* Properties of primers used for the detection of TT virus DNA

| Primer Set | Reference | Name of Primer | Nucleotide Sequence[a] | Length (nt) | Nucleotide Position[b] | Orientation | $T_M$[c] (°C) | $T_A$[d] (°C) | Expected Size of Product (bp) | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| P1 | Nishizawa et al. (1) | RD037 | 5′-GCAGCAGCATATGGATATGT-3′ | 20 | 2008–2027 | Sense | 66.2 | 55 | 270 (1st step) | Nested PCR |
| | | RD038 | 5′-TGACTGTGCTAAAGCCTCTA-3′ | 20 | 2277–2258 | Antisense | 66.2 | | | |
| | | RD051 | 5′-CATACACATGAATGCCAGGC-3′ | 20 | 2061–2080 | Sense | 68.3 | 55 | 197 (2nd step) | |
| | | RD052 | 5′-GTACTTCTTGCTGGTGAAAT-3′ | 20 | 2257–2238 | Antisense | 64.2 | | | |
| P2 | Okamoto et al. Tanaka et al. (2,29) | NG059 | 5′-ACAGACAGAGGAGAAGGCAACATG-3′ | 24 | 1900–1923 | Sense | 73.9 | 60 | 285 (1st step) | Seminested PCR |
| | | NG063 | | | | | | | | |
| | | NG061 | 5′-CTGGCATTTTACCATTTCCAAAGTT-3′ | 25 | 2185–2161 | Antisense | 69.3 | | | |
| | | | 5′-GGCAACATG**Y**T**R**TGGATAGACTGG-3′ | 24 | 1915–1938 | Sense | 72.2–75.6 | | 271 (2nd step) | |
| P2a | Viazov et al. (38) | p59 | 5′-ACAGACAG**R**GG**M**G**R**AGGN**A**A**Y**ATG-3′ | 24 | 1900–1923 | Sense | 70.5–79 | 50 | 281 (1st step) | Seminested PCR |
| | | p63 | 5′-CAT**Y**TT**W**CC**R**TTTCCAAA**R**TT-3′ | 21 | 2181–2161 | Antisense | 59.1–65 | | | Modification of |
| | | p61 | 5′-GG**N**AA**Y**ATG**Y**T**R**TGGATAGACTGG-3′ | 20 | 1915–1938 | Sense | 66.2–74.4 | 57 | 267 (2nd step) | primer set P2 |
| P2b | Takayama et al. (22) | m-NG059 | 5′-ACAGACAGAGG**M**GAAGG**M**AA**Y**ATG-3′ | 24 | 1900–1923 | Sense | 70.5–75.6 | 53 | 285 (1st step) | Seminested PCR |
| | | m-NG063 | 5′-CTGGCAT**Y**TTWCC**R**TTTCCAAA**R**TT-3′ | 25 | 2185–2161 | Antisense | 67.6–72.5 | | | Modification of |
| | | m-NG061 | 5′-GG**M**AA**Y**ATG**Y**T**R**TGGATAGACTGG-3′ | 24 | 1915–1938 | Sense | 68.8–75.6 | 53 | 271 (2nd step) | primer set P2 |
| P3 | Simmonds et al. (34) | A5430 | 5′-CAGACAGAGGAGAAGGCAACATG-3′ | 23 | 1901–1923 | Sense | 73.6 | 55 | 328 (1st step) | Nested PCR, |
| | | A5427 | 5′-TACCA**Y**TTAGCTCTCATTCT**W**A-3′ | 22 | 2228–2207 | Antisense | 63.9–65.7 | | | Modification of |
| | | A8761 | 5′-GG**M**AA**Y**ATG**Y**T**R**TGGATAGACTGG-3′ | 24 | 1915–1938 | Sense | 68.8–75.6 | | 278 (2nd step) | primer set P2 |
| | | A5432 | 5′-CTACCTCCTGGCATTTTACCA-3′ | 21 | 2192–2172 | Antisense | 68.9 | | | |
| P4 | Höhne et al. (37) | TT6 | 5′-ACAGACAGAGGAGAAGGCAA-3′ | 20 | 1900–1919 | Sense | 68.3 | 42 | 329 (1st step) | Nested PCR, |
| | | TT7 | 5′-TACCA**Y**TTAGCTCTCATT-3′ | 18 | 2228–2211 | Antisense | 57.7–59.9 | | | modification of |
| | | TT8 | 5′-AACATG**Y**TATGGATAGACTGG-3′ | 22 | 1918–1938 | Sense | 65.7–67.6 | | 267 (2nd step) | primer set P2 |
| | | TT9 | 5′-CTGGCATTTTACCATTTCCA-3′ | 20 | 2185–2166 | Antisense | 64.2 | | | |
| P5 | Takahashi et al. (21) | T801 | 5′-GCTACGTCACTAACCACGTG-3′ | 20 | 6–25 | Antisense | 70.3 | 60 | 199 | |
| | | T935 | 5′-CT**B**CGGTGTGTAAACTCACC-3′ | 20 | 204–185 | Sense | 68.3–70.3 | | | |

[a]**Y** = T/C; **R** = A/G; **M** = A/C, **B** = G/C/T, **W** = A/T, **N** = A/C/G/T. [b]Nucleotide position according to TT virus isolate GH1 (AF122913). [c]Melting temperature. [d]Annealing temperature applied in PCR.

*Table 2*. Distribution and prevalence of TT virus infection

| | | Healthy Individuals | | Patients | | |
|---|---|---|---|---|---|---|
| Geographical Region | Primer Set[a] | Source of Sera | Number of Cases/ TTV DNA Positive (%) | Diagnosis | Number of Cases/ TTV DNA Positive (%) | References (Year) |
| Japan | P1 | | | Posttransfusion non A-G hepatitis | 5/3 (60) | 1 (1997)[b] |
| Brazil | P2 | Indigenous, rural people | 91/18 (20) | | | 33 (1998) |
| Brazil | P2a | Blood donors | 72/45 (62) | Acute non A-C hepatitis | 52/37 (71) | 46 (1999) |
| Colombia | P2 | Native indians | 140/23 (16.4) | | | 47 (1999) |
| | | General population | 40/4 (10) | | | |
| Congo | P2 | Rural people | 72/32 (44) | | | 33 (1998) |
| Congo | P2 and | Pregnant women | 105/61 (58) | | | 48 (1999) |
| | P3 | Infants (<12 month) | 68/36 (54) | | | |
| Ecuador | P2 | Indigenous, rural people | 96/57 (59) | | | 33 (1998) |
| France | P1 | | | Haemophilia | 95/20 (21) | 40 (1998) |
| | | | | Thalassaemia | 35/6 (17) | |
| France | P3 | | | Anti-HCV-positive, hemodialysis | 44/1 (2.3) | 41 (1999) |
| | | | | Anti-HCV-negative, hemodialysis | 40/1 (2.5) | |
| | | | | Chronic hepatitis C | 40/0 (0) | |
| | | | | Cryptogenic hepatitis | 20/0 (0) | |
| Germany | P2a | | | Decompensated liver cirrhosis | 84/16 (19) | 37 (1998) |
| | | | | Non-A-G-hepatitis | 25/4 (16) | |
| Germany | P2a | Volunteer blood donors | 122/16 (13.1) | End-stage liver disease | 77/34 (44.2) | 38 (1998) |
| | | | | Multiple transfused | 38/21 (55.3) | |
| Germany | P2 | Healthy blood donors | 200/28 (14) | HCV infected | 67/11 (16.4) | 39 (1999) |
| | | Intravenous drug users | 57/11 (19.3) | HGV infected | 47/9 (19.1) | |
| | | | | HIV infected | 25/4 (16.0) | |
| | | | | Fulminant hepatic failure | 18/3 (16.7) | |
| | | | | Hemodialysis patients | 57/11 (19.3) | |
| | | | | Hemophilia patients | 17/3 (17.6) | |
| | | | | Recipients of blood transfusions | 141/28 (19.9) | |
| | | | | After orthotopic liver transplantation | 104/25 (24) | |
| Italy | P2 | Healthy blood donors | 100/22 (22) | Thalassemia, anti HCV$$$ | 93/87 (93.5) | 42 (1999) |
| Italy | P2 | Healthy blood donors | 117/4 (3.4) | Patients with coagulopathy | 34/4 (11.8) | 43 (1999) |
| | | Intravenous drug users | 50/5 (10.0) | Chronic liver disease | 49/6 (12.2) | |
| Japan | P2 | Blood donors | 290/34 (12) | Non-A-G fulminant hepatitis | 19/9 (47) | 2 (1998) |

*Table 2. (Continued)*

| | | Healthy Individuals | | Patients | | |
|---|---|---|---|---|---|---|
| Geographical Region | Primer Set[a] | Source of Sera | Number of Cases/ TTV DNA Positive (%) | Diagnosis | Number of Cases/ TTV DNA Positive (%) | References (Year) |
| | | Intravenous drug users | 35/14 (40) | Non-A-G chronic liver disease | 90/41 (46) | |
| | | | | Hemophilia | 28/19 (68) | |
| | | | | Hemodialysis patients | 57/26 (46) | |
| Japan | P2 | Age-matched controls (mean age 5.3 years) | 187/24 (13) | Haemophilia (virus-inactivated concentrates) | 14/6 (43) | 18 (1998) |
| | | Age-matched controls (mean age 5.3 years) | 311/72 (23) | Haemophilia (not virus-inactivated concentrates) | 58/45 (78) | |
| Japan | P2 | Infant (<2 years) | 24/2 (8.3) | | | 19 (1998) |
| | | Preschool (2–6) | 43/10 (23.3) | | | |
| | | School (7–12) | 88/12 (13.6) | | | |
| | | Adolescent (13–18) | 106/9 (8.5) | | | |
| | | Adult (19–44) | 86/21 (24.4) | | | |
| | | Middle-aged (>45) | 15/5 (33.3) | | | |
| Japan | P2 | | | Rheumatoid arthritis | 46/12 (26) | 20 (1998) |
| Japan | P5 | Healthy adults | 100/92 (92) | | | 21 (1998) |
| | P2 | | 100/23 (23) | | | |
| Japan | P2b | | | Haemophilia | 50/35 (70) | 22 (1999) |
| Japan | P2 | Children (mean age 3.3 years) | 197/10 (5.1) | | | 23 (1999) |
| Japan | P2 | | | Chronic liver disease of unknown etiology | 57/27 (47) | 24 (1999) |
| | | | | Chronic HCV-associated liver disease | 96/17 (18) | |
| Japan | P2 | Healthy blood donors | 50/6 (12) | Hemodialysis patients | 352/113 (32) | 25 (1999) |
| Japan | P2 | Bone marrow donors | 20/2 (10) | Bone marrow recipients | 25/15 (60) | 26 (1999) |
| Japan | P2 | Inhabitants of HCV high endemicity area | 200/116 (58.0) | | | 27 (1999) |
| | | Inhabitants from control area | 200/85 (42.5) | | | |
| Japan | P1 | Healthy volunteers | 100/12 (12) | Acute hepatitis A | 81/4 (4.9) | 28 (1999) |
| | | | | Acute hepatitis B | 30/5 (16.7) | |
| | | | | Acute hepatitis C | 4/1 (25) | |
| | | | | Acute hepatitis of unknown origin | 59/8 (13.6) | |
| Japan | P5 | Newborns (2–3 days, mother TTV positive) | 48/0 (0) | | | 56 (1999) |
| | | Infants (< 1 year) | 6/6 (100) | | | |
| | | Infants (1 year) | 8/7 (87.5) | | | |
| | | Preschoolers (2–6 years) | 20/19 (95) | | | |
| | | School-agers (7–12 years) | 20/18 (90) | | | |
| Korea | P2 | Accepted blood donors | 100/14 (14.0) | Non-B, non-C liver disease | 85/15 (17.6) | 32 (1999) |
| | | Rejected blood donors | 120/23 (19.2) | | | |
| Nigeria | P2 | Rural, periurban people | 63/32 (51) | | | 33 (1998) |

*Table 2. (Continued)*

| | | Healthy Individuals | | Patients | | |
|---|---|---|---|---|---|---|
| Geographical Region | Primer Set[a] | Source of Sera | Number of Cases/ TTV DNA Positive (%) | Diagnosis | Number of Cases/ TTV DNA Positive (%) | References (Year) |
| Pakistan | P2 | Blood donors | 225[c]/[d] (16) | | | 33 (1998) |
| Papua New Guinea | P2 | Indigenous, rural people | 69/51 (74) | | | 33 (1998) |
| Sudan | P2 | Rural people | 70/5 (7) | | | 33 (1998) |
| Thailand | P2 | Blood donors | 105/38 (36) | Chronic liver disease | 127/55 (43) | 29 (1998) |
| Thailand | P2 | Blood donors | 200/14 (7) | Non-A-G chronic liver disease | 50/9 (18) | 30 (1998) |
| | | Pregnant women | 103/7 (6.8) | Hepatocellular carcinoma | 98/9 (9.2) | |
| | | Prostitutes | 31/3 (9.7) | Thalassemia | 80/15 (18.8) | |
| | | Intravenous drug users | 52/17 (32.7) | | | |
| Thailand | P2 | Voluntary blood donors | 200/14 (7) | Hepatocellular carcinoma | 98/9 (9) | 31 (1999) |
| The Gambia | P2 | Rural people | 76/63 (83) | | | 33 (1998) |
| UK | P2 and P3 | Blood donors | 1000/19 (1.9) | Hemophilia | 84/23 (27) | 34 (1998) |
| | | | | Cryptogenic fulminant hepatic failure | 21/4 (19) | |
| UK | P2 | Healthy controls | 30/3 (10) | Chronic liver disease | 72/18 (25) | 35 (1998) |
| UK | P2 and P3 | Low-risk controls | 44/2 (4.5) | Homosexual men (HIV positive) | 23/2 (9) | 36 (1999) |
| | | Prostitutes | 52/7 (13) | Intravenous drug users ($HIV^+$) | 15/1 (7) | |
| | | Homosexual men ($HIV^-$) | 58/6 (10) | | | |
| | | Intravenous drug users ($HIV^-$) | 50/3 (6) | | | |
| USA | P2 | Blood donors | 100/1 (1) | Nontransfused cirrhotics | 25/1 (4) | 44 (1998) |
| | | | | Transfused cirrhotics | 11/2 (18) | |
| | | | | Cryptogenic cirrhosis | 33/5 (15) | |
| | | | | Fulminant hepatic failure | 11/3 (27) | |
| USA | P1 and P3 | Volunteer blood donors | 150/16 (10.7) | Non-A-E hepatitis | 48/1 (2.1) | 45 (1999) |
| | | Commercial blood donors | 148/19 (12.8) | | | |
| | | Intravenous drug users | 87/15 (17.2) | | | |

[a]Properties of the primers are given in Table 1. [b]Detection of TTV, Okamoto et al., 1997 [c]45 pools of 5 [d]25 pools of 5.

different human population groups. TTV infected individuals have been identified all over the world: in Asia (2,18–33), Europe (34–43), North (44,45) and South America (33,46,47), and Africa (33,48). All these studies are based on PCR detection systems, but the technical aspects of the PCR systems vary significantly between the different investigators. These include the sample preparation, PCR primers, and PCR conditions. The properties of the PCR primers employed by the different groups are summarized in Table 1, and their positions within the viral genome are indicated in Fig. 1.

The results of the epidemiological studies do not show a clear picture (Table 2). The prevalence of TTV DNA in the serum of healthy populations varies from 1.9% in Scotland (34) up to 92% in Japan (21). Even in two studies from the same country, e.g. Thailand, DNA seroprevalences range from 7% in one study (30) to 36% in another (29). TTV DNA was detectable in individual patients over a timeperiod of five years (25), and simultaneous infection with different TTV genotypes seems possible (49). TTV-DNA has even been amplified in blood of one of 31 wild African chimpanzees (33).

No standardized TTV PCR detection protocol has been established so far and sensitivity and reliability of the commonly used PCR-assays are suboptimal (21,38,45,48,50). At the moment, none of the available detection systems can be recommended for diagnostic purposes. Most researchers use a semi-nested PCR-system with primers previously published by Okamoto and colleagues or modifications of these primers (Table 1, primer set P2) (2). Some specimens that were analyzed for presence of TTV DNA were TTV DNA negative using primer set P2 (Table 1). However, the same specimens were found to be TTV DNA positive when other primer sets were used (primer sets P1 (45), P3 (34) and P5 (21); Table 1). Takahashi and colleagues reported a DNA seroprevalence of 92% in the general Japanese population using their primer set (P5, Table 1) (21). We found that these primers are not specific for TTV DNA even under stringent conditions but amplify an additional DNA fragment with a size of 159 bp and a DNA sequence that is not homologous to TTV sequences (unpublished observations). It is recommended that all groups using these primers sequence their PCR products to exclude false positive results. The published prevalences are probably significantly underestimated since the applied PCR-systems are not able to amplify all genotypes of TTV under the conditions used. Recently, Tsuda and colleagues developed a method for the detection of antibodies to TTV using TTV that was harvested from feces of a TTV-positive individual (51). Using this method anti-TTV antibodies were detectable in one of six healthy blood donors that were positive for TTV DNA and in 11 out of 38 healthy individuals that were negative for TTV DNA in their serum.

## Modes of Transmission

Although the natural route of transmission of TTV is still unknown, there is clear evidence for a transmission of TTV through blood and blood products. TTV DNA has been detected both in blood, blood products and specifically in peripheral blood mononuclear cells (34,52,53). Populations at risk for parenterally transmitted viruses, e.g. patients with thalassemia (42) or hemodialysis patients (25), have a higher prevalence of TTV infection (Fig. 3). Furthermore, it was reported that patients who had surgery with blood

World wide prevalence of TT virus infection detected by polymerase chain reaction using the oligonucleotide primers NG059, NG063, and NG061

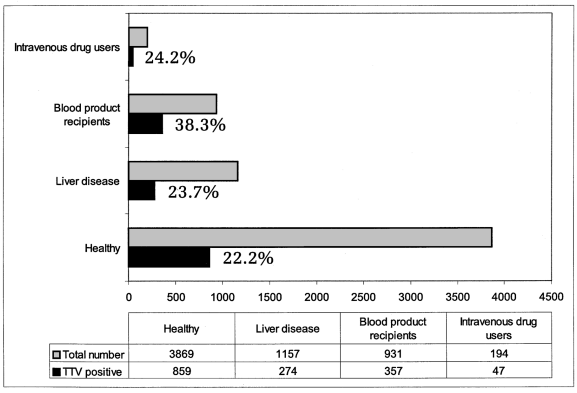


*Fig. 3.* Diagram showing combined data from several studies investigating the prevalence of TTV infection. Only studies using the primers NG059, NG063, and NG061 were included (2,18,19,21,23,25,26,29–33,35,39,42–44,47). ''Liver disease'' indicates patients with chronic liver disease, hepatitis, hepatocellular carcinoma or fulminant hepatic failure. ''Blood product recipients'' indicates patients with coagulopathy, thalassemia, or hemodialysis patients.

transfusions became positive for TTV DNA one to five weeks after the blood transfusion (54,55).

Nevertheless, the parenteral route is probably not the only mode of transmission. In our study young children without a history of blood transfusions are TTV positive, even though their mothers are TTV negative (unpublished results). These results are in agreement with a recent study investigating the incidence and timing of TTV infection in children in a rural area in the Democratic Republic of Congo (48). It was found that a high percentage of children was infected with TTV between three and 12 month after birth. Children of TTV positive mothers had a greater risk of infection, but a great portion of children of TTV negative mothers was also infected. Surprisingly, the genotype of TTV from mother and child was not always identical. Similar results were obtained when the Takahashi primer set (P5; Table 1) (21) was used in a study in Japan. It was found that amniotic fluid, umbilical cord blood and newborn's sera from TTV positive women were negative for TTV DNA, whereas mother's milk was TTV DNA positive. In contrast, nearly all sera from children between two month and 12 years of age were positive for TTV DNA (56). This data and the fact that TTV DNA can be detected in the feces of infected individuals (57) suggest that it may be possible to attract TTV infection from environmental sources.

## Pathogenicity

The observed high prevalence of TTV infection in the healthy population raises the question whether an infection with TTV has pathogenic effects on the human host at all (58,59). However, several reports state a correlation of TTV-titer and elevation of serum alanine transferase (ALT) levels (1,26,54). The virulence and the pathogenic potential of the different genotypes or strains of TTV is still unclear and requires further investigation. The only animal experiment published is the experimental infection of two chimpanzees with TTV (4). The chimpanzees did not show any biochemical or histological evidence of hepatitis.

## Conclusions and Perspectives

The discovery of TTV as a viral agent and particularly the identification of a high percentage of infected carriers in the healthy human population raises the following questions:

Firstly, what is the origin and molecular relatedness of TT virus. Secondly, what is the significance of TTV as a human pathogen. And thirdly, what are the exact molecular mechanisms of viral replication.

To answer these questions it will be necessary to determine the primary structure and the coding capacity of several TTV patient isolates. This data will then allow the optimization of PCR primer sets leading to a more standardized approach in diagnostics and to molecular epidemiological studies that can discern between different genotypes of TTV. Reliable primary structure data will make it possible to develop serological assays for TTV antibody detection using recombinant viral proteins. Another important goal for TTV research should be the development of a cell culture or animal model that will allow molecular studies of viral transcription and replication and the propagation of the virus.

In general we believe that it is at this point more important to focus on basic research rather than on the accumulation of more and more epidemiological data that is based on unreliable detection assays. More basic research will ultimately lead to an understanding of the significance of TTV as a human pathogen.

## Notes

1. The term ''transfusion-transmitted virus'' introduced in later reports is misleading and should no longer be used (60).
2. He, Z. and collegues reported a TTV genome sequence from China under Genbank accession number AF116842 as unpublished results in May 1999.
3. GenBank accession numbers CAV: D10068, U65414, U66304, AB027470; BFDV: AF080560, AF071878 PCV:U49186, AF117753, AF112862, AF109399, AF109398, AF012107, AF085695, AF086836, AF086835, AF086834, AF027217, U49186, AF055394, AF055393, AF055392, AF055391, AF071879, AJ223185.
4. References used in this review include all TTV specific literature published until May 31, 1999 (NCBI-NIH public medline (Pubmed)).

## Note Added in Proof

During the reviewing of this article, the following important progress in TT virus research has been made:

i) several genomic TT virus DNA nucleotide sequences have been determined (61–63) (GenBank accession numbers AF122914 to AF122921, AB025946, AB017613);

ii) new oligonucleotide primers were used for the detection of TTV(64,65);
iii) TTV has been detected in farm animals and non-human primates (66).

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