

Research of Torque Teno Virus (TTV) in Serum and Total Blood of Brazilian Non-Human Primates and in Chicken Plasma (*Gallus gallus domesticus*) by the PCR N22 Region

Investigación de Torque Teno Virus (TTV) en Suero y Sangre de Primates No Humanos Brasileños y en Plasma de Pollo (*Gallus gallus domesticus*) por PCR en la Región N22

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SUMMARY: Torque teno virus (TTV) is a recently discovered DNA virus that was originally isolated from a Japanese patient (initials, TT) with post-transfusion hepatitis of unknown aetiology. TTV is an circular DNA virus classified recently together with related Torque teno minivirus, into a new genus called *Anellovirus*. Infection TTV has been detected in a range of non-human primates as well as domestic animals. The purpose of this study was to search TTV in the serum and total blood of Brazilian monkeys and in plasma of domestic chickens by seminested PCR of coding region (N22), followed by a genomic sequence and phylogenetic analysis. No serum sample was amplified. TTV DNA was detected in total blood from 3 (4%) out of 75 brown-capuchin (*Cebus apella*) and from 1 (25%) out of 4 golden-headed lion-tamarin (*Leontopithecus chrysomelas*). Phylogenetic analysis revealed that one sample showed similarity with one sequence of the cotton top tamarin (*Saguinus oedipus*) (So-TTV2) and with one of the douroucoulis (*Aotes trivirgatus*) (At-TTV3). Two samples showed similarity with a human Torque Teno Mini Virus (TLMV). The other sample clustered with one sequence of the chimpanzee (Pt-TTV6) and with the human TTV strain TA278. The plasma chicken samples tested were all negative. The amino acid sequences reported in this study are the first obtained in Brazil from total blood of non-human primates naturally infected by TTV.

KEY WORDS: Torque teno virus; Non-humans primates; Coding region; Amino acid sequencing.

INTRODUCTION

Torque Teno virus (TTV) is a recently discovered DNA virus that was originally isolated from a Japanese patient (initials, TT) with post-transfusion hepatitis of unknown aetiology (Nishizawa *et al.*, 1997). Since discovery of TTV, studies have been published describing the prevalence of TTV infection in people with acute or chronic hepatitis as well as in blood donors and drug users and also in health persons (Biagini, 1998; Mac Donald *et al.*, 1999). TTV is an unenveloped, single-stranded, and circular DNA virus with 30-32 nm of diameter (Miyata *et al.*, 1999; Mushahwar *et al.*, 1999; Itoh *et al.*, 2000), classified recently

together with the related Torque Teno Minivirus, into a new genus called *Anellovirus* (Biagini *et al.*, 2005).

TTV is transmitted parenterally by transfusion (Okamoto *et al.*, 1999), by enteric transmission demonstrated experimentally in chimpanzees (Luo & Zhang, 2001; Tawara *et al.*, 2000), and by vertical transmission (Saback *et al.*, 1999). Also has been detected in saliva, on throat swabs, semen, tears, breast milk, hair, skin and raw (Saback *et al.*; Goto *et al.*, 2000; Inami *et al.*, 2000; Osiowy & Sauder, 2000; Al-Moslih *et al.*, 2007). Infection TTV has been detected in

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a range of non-human primates (Leary *et al.*, 1999; Verschoor *et al.*, 1999; Abe *et al.*, 2000; Cong *et al.*, 2000; Okamoto *et al.*, 2000a,b; Noppornpanth *et al.*, 2001; Thom *et al.*, 2003; Barnet *et al.*, 2004; Pujol *et al.*, 2005) as well as domestic animals, such as dogs, cats, swines, bovines, chickens, ovines, tupaia (tree shrews), wild boar (*Sus scrofa*) and camels (Leary *et al.*; Okamoto *et al.*, 2001a; 2002; Mc Keown *et al.*, 2004; Bigarré *et al.*, 2005; Niel *et al.*, 2005; Martelli *et al.*, 2006; Martinez *et al.*, 2006; Al-Moslih *et al.*).

Study of experimental transmission points out that the chimpanzees are susceptible to TTV infection and that can be cross infected with human (Mushahwar *et al.*; Okamoto *et al.*, 2000a, b).

TTV genome includes two regions: a coding region (N22) and an untranslated region (UTR). The UTR is located at nt 3075-3853 and nt 1-352 occupying approximately 30% of the genome (Okamoto *et al.*, 1999; Ukita *et al.*, 2000). Coding region consist of 6 ORFs with open reading frames (ORF1-ORF6) (Yokoyama *et al.*, 2002). The N22 amplifies a fragment in open reading frame 1 (ORF1) and represents approximately 220 nts of a genome that usually encompasses more than 3800 nts (Miyata *et al.*; Mushahwar *et al.*). Consequently, almost every phylogenetic analysis of TTV is focused on this genomic region (Lemey *et al.*, 2002).

The significant diversity of the TTV genome allows for its classification into more than 40 genotypes which cluster in five clearly distinct phylogenetic groups that are designated 1 to 5 (Okamoto & Mayumi, 2001; Hino, 2002; Peng *et al.*, 2002; Biagini *et al.*).

Other members of this virus family (SANBAM, YONBAN, TLMV (Torque teno Minivirus), SENV (SEN virus) and PMV (PM virus), presenting some genomic divergences have been related (Cong *et al.*; Hallet *et al.*, 2000; Khudyakov *et al.*, 2000; Takahashi *et al.*, 2000b; Diniz-Mendes *et al.*, 2004).

The purpose of this study was to search TTV in the serum and blood of Brazilian monkeys and plasma of domestic chickens (*Gallus gallus domesticus*) by PCR of coding region (N22), followed by a genomic sequence and phylogenetic analysis.

MATERIAL AND METHOD

Animals: Were collected from August, 2000 to February, 2001, 90 serum samples and 70 total blood samples from various non-human primates, both sexes, including 26

Callithrix jacchus, 3 *Callithrix kullii*, 4 *Leontopithecus crysomelas*, 75 *Cebus apella*, 19 *Callithrix penicillata*, 2 *Callithrix sp.*, 5 *Alouata caraya*, 5 *Alouata fusca*, 1 *Saguinus m. niger*, 1 *Callithrix humeralifer*, 3 *Callithrix geoffroyi*, 1 *Aotus trivirgatus*, 1 *Calimico goeldi*, 3 *Saguinus m. midas*, 1 *Lagothrix lagotricha*, 1 *Erythrocebus pata*, 2 *Ateles b. marginatus*, 1 *Ateles peniscus*, 4 *Saimiri sciureus*, 1 *Saimiri fuscicollis*, 1 *Leontopithecus chrysopygus*. These species are usually found in the Amazon Forest and Atlantic Rainforest (Mata Atlantica), in Brazil. These animals came from donations or rescues and were apprehended and sent to several ecological and Zoological Parks in São Paulo, Brazil where they were maintained in captivity. A total of 117 samples of chicken plasma (*Gallus gallus domesticus*) of the following lineages: white, brown leghorn and hens of yard, of both sexes from different poultry farms in the State of São Paulo, SP, Brazil, were collected from May to September 2001. Samples were kept at -80°C until testing.

Extraction of nucleic acids and amplification by PCR: Blood DNA was extracted using a DNA Extraction Kit (Qiagen) from 100 µl total blood. DNA was resuspended in 100 µl buffer. Serum DNA (50 µl) was mixed with 100 µl Dnazol and 3 µl Dextran T500 (1mg/µl). The DNA was precipitated with isopropanol and resuspended in 25 µl distilled water.

Heminested N22 PCR: In the first round, outer primers NG059 and NG063 (sequences 5' ACA GAC AGA GGA GGG AAC ATC – 3' and 5' – CTG GCA TTT TAG CAT TTC CAA AGTT – 3') were used.

PCR was carried out in 50 µl volume, using 5 µl of DNA, 10 pmol of NG059 and NG063 primers, 1.5 mM MgCl₂ and 5U Taq polymerase. In the second round, semi-inner primer NG061 (sequence 5' – GGC AAG ATG YTR TGG ATA GAC TGG – 3') and the outer primer NG063 were used. Five µl of the first PCR product and 10 pmoles/µl of NG061 and NG063 primers were used for the second PCR. Amplifications conditions were 94°C 30s, 55°C 30s, 72°C 30s, 35 cycles, with a final extension at 72°C 7 min.

The amplification products of the first PCR round were 286 pb, and those of the second round were 271bp. It was considered positive for the TTV control, human serum from a TTV positive patient, and negative, ultra pure sterile water, free from inhibitors or contaminants, which could avoid amplification of PCR products. Amplified products were separated by electrophoresis through a 2% agarose gel containing ethidium bromide, and visualized under UV light.

Sequencing. Sequence reactions were performed with the ABI Prism Big Dye Terminator Cycle sequencing Ready Reaction Kit (Applied Biosystems). Cycle sequencing was

Phylogenetic analysis revealed that one sample showed similarity with one sequence of the cotton top tamarin (*Saguinus oedipus*) (So-TTV2) and with one of the douroucoulis (*Aotes trivirgatus*) (At-TTV3). Two samples showed similarity with a human Torque Teno Mini Virus (TLMV). The other sample clustered with one sequence of the chimpanzee (Pt-TTV6) and with the human TTV strain TA278 (Fig. 2).

Gallus gallus domesticus. The 117 plasm samples tested by the semi-nested PCR reaction for the N22 region were all negative.

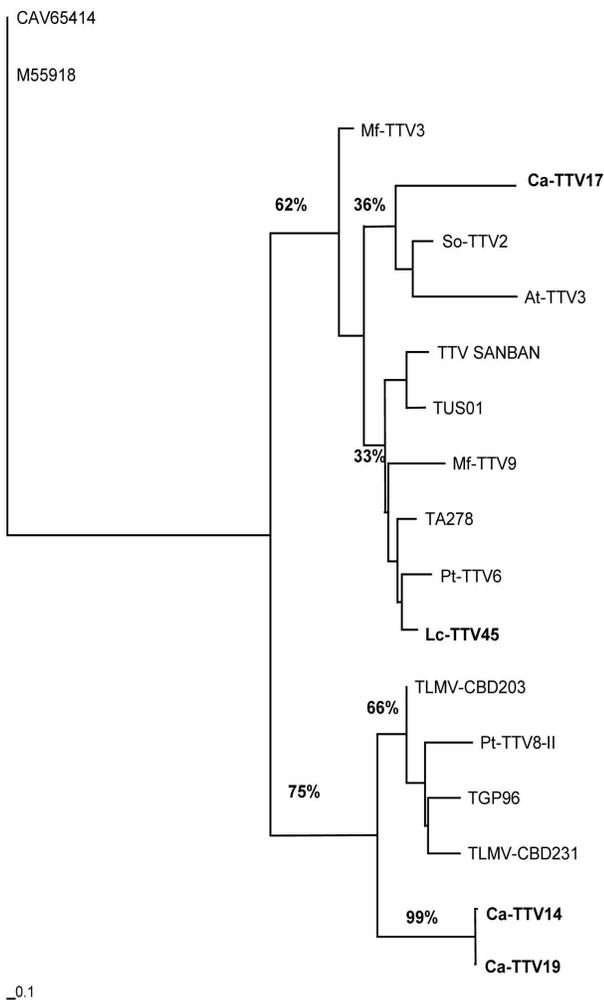


Fig. 2. Phylogenetic relationship between TTV-N22 amino acid sequences obtained from Brazilian non-human primates and sequences isolated from humans, non-humans primates and chickens. The phylogenetic trees were constructed by the neighbour-joining method (Saitou & Nei). Isolates determined in this study are presented in bold. The database-derived isolates and their accession numbers is given in the legend to Fig. 1.

DISCUSSION

The diagnostic of TTV infection is frequently realized by DNA amplification by PCR utilizing primers designed or by coding region of the ORF 1 (N22) or by non-coding region (UTR) of the viral genome (Ott *et al.*, 2000).

The phylogenetic analysis of the TTV have been based on a small genome region that exhibits extensive sequence heterogeneity (Lemey *et al.*).

Our results obtained by the semi-nested PCR reaction for the N22 region revealed amplification in 4 (5.7%) out of the 70 samples of total blood of new world non-human primates. However, no amplification was observed in 117 serum of the animals submitted to the same reaction.

On the contrary, other studies did not obtain positivity by the PCR of the N22 region, in samples of non-human primates of the old and new world (Abe *et al.*; Pujol *et al.*), not even in pig, cat and dog samples (Okamoto *et al.*, 2002).

Studies in the literature have demonstrated variations in positivity percentage in samples of the old world non-human primates. Nopporpanth *et al.* detected 9 (13.4%) of 67 gibbons, Thom *et al.* 1 (20%) of 5 chimpanzees, Romeo *et al.* (2000) 2 (25%) out of 8 naive chimpanzees, Verschoor *et al.* 60 (48.%) out of 123 chimpanzees e 4 (66.%) out of 6 pygmy chimpanzees, Barnett *et al.*, 4 (80%) of 5 chimpanzees of the Congo community and 3 (100%) of 3 chimpanzees of the Sonso community of the Budongo Forest Reserve of Uganda.

Using specific primers for each species of chimpanzee, Okamoto *et al.* (2000a) detected 57% in 49 out of 104 chimpanzees, but they did not obtain positivity in any sample that they have tested with new world non-human primates.

No other study in the literature has detected the presence of DNA TTV in new world non-human primates by the N22-PCR.

Okamoto *et al.* (2000a) e Leary *et al.*, however, detected DNA TTV in new world monkeys by PCR of the non-coding region (UTR), since the primers used in the N22-PCR only allow the amplification of a restricted number of TTV genotypes (Bendinelli *et al.*, 2001; Vasconcelos *et al.*, 2002).

In this study DNA TTV was detected in 3 samples of *Cebus apella* and in 1 sample of *Leonthopithecus crysomelas*. Abe *et al.* however, had detect DNA TTV in none of the 9 *Cebus apella* samples submitted to N22-PCR .

Cebus apella is the greatest specie, largely distributed in South America, present in all Amazon Forest, swamp, woodsy pasture, upland caatinga and Atlantic Rain Forest (Silva Júnior, 2002).

In our study one sequence of the *Cebus apella* clustered with one strain isolated from *Saguinus oedipus* (So-TTV2) and other with from *Aotes trivirgatus* (At-TTV3) of the Okamoto *et al.* (2000b).

Okamoto *et al.* (2000b) performed by cloned from UTR-PCR products representing the full-length TTV genomes obtained from sera of humans and non-human primates that showed common genomic organization with two open reading frame (ORFs), designated ORF1 and ORF2 and their sequences were determined. In accordance with the author, these sequences bifurcated from the same branch harboring Japanese macaque TTV, reflecting a close relationship of these lower classes non-human primates TTVs.

Our results showed that one sequence clustered with a human TLMV (Torque Teno Mini Virus) sequence.

Furthemore, TTV and TLMV DNA sequences have been found in non-human primates and farm animals (Verschoor *et al.*, 1999; Abe *et al.*, 2000; Leary *et al.*, 1999; Okamoto *et al.*, 2000 a, b; Romeo *et al.*, 2000).

TLMV is a unique virus with a smaller genome that may constitute a genetic evolutionary link between chicken anemia virus and TTV (Takahashi *et al.*, 2000a).

In this study, 1 amplified sample (*Cebus apella*) was grouped with one strain of chimpanzee (Pt-TTV6) (Okamoto *et al.*, 2000b) and with the TA278 human prototype strain.

A close proximity was observed, between the gibbon virus and those detected in Thai individuals, whereas that, chimpanzee strains were phylogenetically more remote (Noppornpanth *et al.*).

According to Verschoor *et al.* TTV from pygmy chimpanzees and the common chimpanzees closely to viruses from human TTV from the common chimpanzees subspecies *Pan troglodytes verus* and *Pan troglodytes schweinfurthii* cluster together, suggesting an ancient host-pathogen relationship before sub speciation 1.6 million years ago and TTV of common and pygmy chimpanzees may have been acquired by these animals in different zoonotic events not longer than 2.5 million years ago.

Abe *et al.* reported one study that all TTV isolates obtained from simians (s-TTV) (old world non-human primates) were clearly distinct from TTV found in humans.

Inami *et al.* stated that a strain CH65-1 in ORF-1 region showed only 35% identity to the prototype TA278 human isolate at the amino acid level.

The results of our research showed that the sequence of DNA TTV obtained of the *Cebus apella* (Ca-TTV17) were close to human TTV of the genotype 1.

In the same manner, gibbons sequences (Noppornpanth *et al.*) and in pygmy chimpanzees Okamoto *et al.* (2000a, b) were grouped in genotype 1.

Verschoor *et al.* however, reported that TTV sequences from pigmy chimpanzees are closely related to viruses from human genotypes 2 and 3, while that sequences obtained from common chimpanzees were genotypes 5 and 6, the latter only at the protein level.

The phylogenetic analyses of the ORF1 nucleotide consensus sequences suggested that TTV recovered from two chimpanzees (CH1304 and CH 1545) represented two new genotypes (Romeo *et al.*).

Simian TTV (s-TTV) was further divided into three genotypes and showed type 1 as the major genotype (Abe *et al.*). In this study, it was not possible to detect DNA TTV by N22-PCR in 117 samples chicken plasma.

Only two reports refer to TTV research in chicken by PCR of the non-coding region (UTR). Thom *et al.* had investigated the presence of virus in 29 chicken samples, as well as the 20 samples from cows, 20 from sheep and 20 from goats, but all were PCR-UTR negative. Also, Leary *et al.* detected the virus in various farm animal species (chickens, pigs, cows and sheep) and verified that 19% of chickens were positive by PCR-UTR, whose sequences clustered with human TTV. This different result are due to the fact of using primers deduced from a coding region sequence (N22).

The primers of the N22 region only allow the amplification of a restricted number of TTV genotypes (Bendinelli *et al.*; Vasconcelos *et al.*). Our results indicate that in spite of the TTV has been detected worldwide in wide scale in various animal species, in Brazil, however, their presence in new world non-human primates, in captivity and in chickens occurs in low number.

All the animals were clinically healthy, but very little is known, however, about the TTV infection of new world primates. The virus might have been transmitted from human handler to animals in the process of feeding or the wise handling the monkeys.

According Luo & Zhang TTV is transmitted both by blood and enteric routes and perhaps in areas with inadequate hygienic standards, the virus is predominantly spread via the fecal-oral route.

The amino acid sequences reported in this study are the first obtained in Brazil from total blood of non-human primates naturally infected by TTV. The amino acid identity

between human and non-human primates sequences in genotype 1 observed in this study supports the evidence that TTV is a zoonotic agent.

Further studies to evaluate the pathology, genetic diversity and host range, transmission, and persistence of TTV infection, in human and animal species will be conducted in order to outline considerable conclusions.

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RESUMEN: Torque teno virus (TTV) es un virus de ADN recientemente descubierto que fue inicialmente aislado de un paciente japonés (iniciales TT) después de la transfusión de hepatitis de etiología desconocida. TTV es un virus de ADN circular recientemente clasificado junto con los torque teno minivirus, en un nuevo género llamado *Anellovirus*. La infección de TTV se ha detectado en una serie de primates no humanos, así como animales domésticos. El objetivo de este estudio fue buscar TTV en el suero y sangre total de monos de Brasil y en el plasma de pollos domésticos, por seminested PCR de la región de codificación (N22), seguido de una secuencia genómica y el análisis filogenético. Las muestras que no eran suero fueron amplificadas. TTV DNA se detectó en sangre total de 3 (4%) de un total de 75 capuchinos de cabeza dura (*Cebus apella*) y de 1 (25%) de un total de 4 tí- león de cabeza dorada (*Leontopithecus chrysomelas*). El análisis filogenético demostró que una muestra presentaba similitud con una secuencia de *Saguinus Edipo* (So-TTV2) y con una de *Aotes trivirgatus* (A-TTV3). Dos muestras mostraron similitud con un torque teno mini virus (TLMV) humano. La otra muestra agrupada con una secuencia de los chimpancés (PT-TTV6) y con el TTV humano cepa TA278. El análisis de las muestras de plasma de pollo fueron negativas. Las secuencias de aminoácidos que se reportan en este estudio son las primeras obtenidas en Brasil de sangre de primates no humanos infectados naturalmente por TTV.

PALABRAS CLAVE: Torque teno virus; Primates no humanos; Región de codificación; Secuencia de aminoácidos.

REFERENCES

- Abe, K.; Inami, T.; Ishikawa, K.; Nakamura, S. & Goto, S. TT virus infection in nonhuman primates and characterization of the viral genome: identification of Simian TT virus isolates. *J. Virol.*, 74(3):1549-53, 2000.
- Al-Moslih, M. I.; Perkins, H. & Hu, Y. W. Genetic relationship of Torque Teno virus (TTV) between humans and camels in United Arab Emirates (UAE). *J. Med. Virol.*, 79:188-91, 2007.
- Barnett, O. E.; Worobey, M.; Homes, E. C. & Cooper, A. Detection of TT virus among chimpanzees in the wild using a noninvasive technique. *J. Wildl. Dis.*, 40:230-7, 2004.
- Bendinelli, M.; Pistello, M.; Maggi, F.; Fornai, C.; Freer, G. & Vatteroni, M. L. Molecular properties, biology and clinical implications of TT virus, a recently identified widespread infectious agent of humans. *Clin. Microbiol. Rev.*, 14(1):98-113, 2001.
- Biagini, P. Presence of TT virus in French blood donors and intravenous drug users. *J. Hepatol.*, 29:684-5, 1998.
- Biagini, P.; Todd, D. & Bendinelli, M. *Anellovirus*. In: Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses. Fauquet, C. M.; Mayo, M. A.; Maniloff, J.; Desselberger, U. & Ball, L. A. Eds. London, Academic Press, 2005. pp. 335-41.
- Bigarré, L.; Beven, V.; Boissédon, C.; Grasland, B. O.; Rose, N. O.; Biagini, P. O. & Jestin, A. Pig anelloviruses are highly prevalent in swine herds in France. *J. Gen. Virol.*, 86:631-5, 2005.
- Cong, M.; Nichols, B.; Dou, X.; Spelbring, J. E.; Krawczynski, K.; Fields, H. A. & Khudyakov, Y. E. Related TT viruses in chimpanzees. *Virology*, 274:343-55, 2000.
- Diniz-Mendes, L.; Devale, S. & Niel, C. Genomic characterization of a Brazilian TT virus isolate closely related to SEN Virus- F. *Mem. Inst. Oswaldo Cruz.*, 99:301-6, 2004.
- Felsenstein, J. PHILIP, version 3.5. Dept. of Genetics, University of Washington, Seattle, USA, 1993. (Distributed by the author at <http://evolution.genetics.washington.edu/phyip.html>).
- Goto, K.; Sugiyama, K.; Ando, T.; Mizutani, F.; Terabe, K.; Tanaka, K.; Nishiyama, M. & Wada, Y. Detection rates of TT virus DNA in serum of umbilical cord blood, breast milk and saliva. *Tohoku. J. Exp. Med.*, 191(4):203-7, 2000.
- Hall, T. A. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.*, 41:95-8, 1999.
- Hallett, R. L.; Clewley, J. P.; Bobet, F.; McKiernan, P. J. & Teo,

- C. G. Characterization fo a highly divergent TT virus genome. *J. Gen. Virol.*, 81:2273-2279, 2000.
- Hamooleh, G.; Cross, G. & Dixon, R. J. DNA cloning and nucleotide sequencing of Australian chicken anaemia virus (U65414) (unpublished), 1996.
- Hijikata, M.; Takahashi, K. & Mishiro, S. Complete circular DNA genome of a TT virus variant (isolate name SANBAN) and 44 partial ORF2 sequences implicating a great degree of diversity beyond genotypes. *Virology*, 260:17-22, 1999.
- Hino, S. TTV, a new human virus with single stranded circular DNA genome. *Rev. Med. Virol.*, 12(3):151-8, 2002.
- Inami, T.; Obara, T.; Moriyama, M.; Arakawa, Y. & Abe, K. Full-length nucleotide sequence of a simian TT virus isolate obtained from a chimpanzee: evidence for a new TT virus-like species. *Virology*, 277:330-5, 2000.
- Itoh, J.; Takahashi, M.; Fukuda, M.; Shibayama, T.; Ishikawa, T.; Tsuda, F.; Tanaka, T.; Nishizawa, T. & Okamoto, H. Visualization of TT virus particles recovered from the sera and feces of infected humans. *Bioch. Bioph. Res. Comm.*, 279:718-24, 2000.
- Khudyakov, Y. E.; Cong, M. E.; Nichols, B.; Reed, D.; Dou, X. G.; Viazov, S. O.; Chang, J.; Fried, M. W.; Roggendorf, M. & Fields, H. A. Sequence heterogeneity of TT virus and closely related viruses. *J. Virol.*, 74(7):2990-3000, 2000.
- Leary, T. P.; Erker, J. C.; Chalmers, M. L.; Desai, S. M. & Mushahwar, I. K. Improved detection systems for TT virus reveal high prevalence in humans, non-humans primates and farm animals. *J. Gen. Virol.*, 80:2115-20, 1999.
- Lemey, P.; Salemi, M.; Bassit, L. & Vandamme, A-M. Phylogenetic classification of TT virus groups based on the N22 region is unreliable. *Virus Res.*, 85(4):47-59, 2002.
- Luo, K. & Zhang, L. Enteric transmission of transfusion-transmitted virus. *Chin. Med. J.*, 114:1201-4, 2001.
- Mac Donald, D. M.; Scott, G. R.; Clutterbuck, D. & Simmonds, P. Infrequent detection of TT virus infection in intravenous drug users, prostitutes, and homosexual men. *J. Infect. Dis.*, 179:686-9, 1999.
- Martelli, F.; Caprioli, A.; Di Bartolo, I.; Cibir, V.; Pezzotti, G. Ruggeri, F. M. & Ostanello, F. Detection of swine Torque teno virus in Italian pig herds. *J. Vet. Med.*, B53:234-8, 2006.
- Martinez, L.; Kekarainen, T.; Sibila, M.; Ruiz-Fons, F.; Vidal, D. & Segales, J. Torque teno virus (TTV) is highly prevalent in the European wild boar (*Sus scrofa*). *Vet. Microbiol.*, 118(3-4):223-9, 2006.
- Mc Keown, N. E.; Fenaux, M.; Halbur, P. G. & Meng, X. J. Molecular characterization of porcine TT virus, and orphan virus, in pigs from six different countries. *Vet. Microbiol.*, 104:113-7, 2004.
- Miyata, H.; Hsunoda, H.; Kazi, A.; Yamada, A.; Khan, M. A.; Muakami, J.; Kamahora, T.; Shiraki, K. & Hino, S. Identification of a novel GC-rich 113 nucleotide region to complete the circular single-stranded DNA genome of TT virus, the first human circovirus. *J. Virol.*, 73:3582-6, 1999.
- Mushahwar, I. K.; Erker, J. C.; Murchoff, A. S.; Leary, T. P.; Simons, J. N.; Birkenmeyer, L. G.; Chalmers, M. L.; Pilot-Matias, T. J. & Desai, S. M. Molecular and biophysical characterization of TT virus: evidence for a new virus family infecting humans. *Proc. Natl. Acad. Sci.*, 96:3177-82, 1999.
- Niel, C.; Diniz-Mendes, L. & Devalle, S. Rolling-circle amplification of Torque teno virus (TTV) complete genomes from human and swine sera and identification of a novel swine TTV genogroup. *J. Gen. Virol.*, 86:1343-7, 2005.
- Nishizawa, T.; Okamoto, H.; Konishi, K.; Yoshizawa, H.; Miyakawa, Y. & Mayumi, M. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. *Biochem. Biophys. Res. Commun.*, 241:92-7, 1997.
- Noppornpanth, S.; Chingai, T.; Ratanakorn, P. & Poovorawan, Y. TT virus infection in gibbons. *J. Vet. Med. Sci.*, 63:663-6, 2001.
- Noteborn, M. H.; de Boer, G. F.; van Roozelaar, D. J.; Karreman, C.; Kranenburg, O.; Vos, J. G.; Jeurissen, S. H.; Hoeven, R. C.; Zantema, A.; Koch, G.; van Ormondt, H. & van der Eb, A. J. Characterization of cloned chicken anemia virus DNA that contains all elements for the infectious replication cycle. *J. Virol.*, 65:3131-9, 1991.
- Okamoto, H.; Nishizawa, Ukita, M.; Takahashi, M.; Fukuda, M.; Lizuka, H.; Miyakawa, Y. & Mayumi, M. The entire nucleotide sequence of a TT virus isolate from the United States (TUS 01): comparison with reported isolates and phylogenetic analysis. *Virology.*, 259:437-48, 1999.
- Okamoto, H.; Fukuda, M.; Tawara, A.; Nishizawa, T.; Itoh, Y.; Ayasaka, I.; Tsuda, F.; Tanaka, T.; Miyakawa, Y. & Mayumi, M. Species-specific TT virus and cross-species infection in nonhuman primates. *J. Virol.*, 74(3):1132-9, 2000a.
- Okamoto, H.; Nishizawa, T.; Tawara, A.; Peng, Y.; Takahashi, M.; Kishimoto, J.; Tanaka, T.; Miyakawa, Y. & Mayumi, M. Species-specific TT viruses in humans and nonhuman primates and their phylogenetic relatedness. *Virology*, 277(2):368-78, 2000b.

- Okamoto, H. & Mayumi, M. TT virus: virological and genomic characteristics and disease associations. *J. Gastroenterol.*, 36:519-29, 2001.
- Okamoto, H.; Nishizawa, T.; Takahashi, M.; Tawara, A.; Peng, Y.; Kishimoto, J. & Wang, Y. Genomic and evolutionary characterization of TT virus (TTV) in Tupaias and comparison with species-specific TTVs in humans and non-human primates. *J. Gen. Virol.*, 82:2041-50, 2001a.
- Okamoto, H.; Takahashi, M.; Nishizawa, T.; Tawara, A.; Fukai, K.; Muramatsu, U.; Naito, Y. & Yoshikawa, A. Genomic characterization of TT viruses (TTVs) in pigs, cats and dogs and their relatedness with species-specific TTVs in primates and tupaia. *J. Gen. Virol.*, 83:1291-7, 2002.
- Osiowy, C. & Sauder, C. Detection of TT virus in human hair and skin. *Hepatology*, 16:155-62, 2000.
- Ott, C.; Duret, L.; Chemin, I.; Trépo, C.; Mandrand, B. & Komurian-Pradel, F. Use of a TT virus ORF 1 recombinant protein to detect anti-TT virus antibodies in human sera. *J. Gen. Virol.* 81:2949-58, 2000.
- Peng, Y. H.; Nishizawa, T.; Takahashi, M.; Ishikawa, T.; Yoshikawa, A. & Okamoto, H. Analysis of the entire genomes of thirteen TT virus variants classifiable into the fourth and fifth genetic groups, isolated from viremic infants. *Arch. Virol.*, 147:21-41, 2002.
- Pujol, F. H.; Mejías, E.; Loreiro, C. L.; Ludert, J. E.; Liprandi, F. & Pernalet, J. M. Infection with transfusion-transmitted virus (TTV) in humans and other primates in Venezuela. *Ann. Trop. Med. Parasitol.*, 99:173-80, 2005.
- Roderic, D. M. TREEVIEW. Glasgow, Scotland: University of Glasgow, (Distributed by the author at <http://taxonomy.zoology.gla.ac.uk/rod/treeview.htm>), 2001.
- Romeo, R.; Hegerich, P.; Emerson, S. U.; Colombo, M.; Purcell, R. H. & Bukh, J. High prevalence of TT virus (TTV) in naive chimpanzees and in hepatitis C virus-infected humans: frequent mixed infections and identification of new TTV genotypes in chimpanzees. *J. Gen. Virol.*, 81:1001-7, 2000.
- Saback, F. L.; Gomes, S. A. S.; Paula, V. S.; Da Silva, R. R. S.; Lewis-Ximenez, L. L. & Niel, C. Age specific prevalence and transmission of TT virus. *J. Med. Virol.*, 59:318-22, 1999.
- Saitou, N. & Nei, M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4:406-25, 1997.
- Silva Júnior, J. S. Taxonomy of capuchin monkeys, *Cebus Erxleben*. *Neotrop. Primates*, 2(1):29, 2002.
- Takahashi, K.; Iwasa, Y.; Hijikata, M.; Mishiro, S. Identification of a new human DNA virus (TTV-like mini virus, TLMV) intermediately related to TT virus and chicken anemia virus. *Arch. Virol.*, 145:979-93, 2000a.
- Takahashi, K.; Hijikata, M.; Samokhvalov, E. I. & Mishiro, S. Full or near full length nucleotide sequences of TT virus variants (types SANBAN and YONBAN) and TT virus-like mini virus. *Intervirology*, 43(2):119-23, 2000b.
- Tawara, A.; Akahane, Y.; Takahashi, M.; Nishizawa, T.; Ishikawa, T. & Okamoto, H. Transmission of human TT virus of genotype 1a to chimpanzees with fecal supernatant or serum from patients with acute TTV infection. *Biochem. Biophys. Res. Commun.*, 278(2):470-6, 2000.
- Thom, K.; Morrison, C.; Lewis, J. C. M. & Simmonds, P. Distribution of TT virus (TTV), TTV-like minivirus, and related viruses in humans and nonhuman primates. *Virology*, 306:324-33, 2003.
- Ukita, M.; Okamoto, H.; Nishizawa, T.; Tawara, A.; Takahashi, M.; Iizuka, H.; Miyakawa, Y. & Mayumi, M. The entire nucleotide sequences of two distinct TT virus (TTV) isolates (TJN01 and TJN02) remotely related to the original TTV isolates. *Arch. Virol.*, 145:1543-59, 2000.
- Vasconcelos, H. C.; Cataldo, M. & Niel, C. Mixed infections of adults and children with multiple TTV-like mini virus isolates. *J. Med. Virol.*, 68(2):291-8, 2002.
- Verschoor, E. J.; Langenhuijzen, S. & Heeney, J. L. TT viruses (TTV) of non-human primates and their relationship to the human TTV genotypes. *J. Gen. Virol.*, 80:2491-9, 1999.
- Yokoyama, H.; Yassuda, J.; Okamoto, H. & Iwakura, Y. Pathological changes of renal epithelial cells in mice transgenic for the TT virus ORF1 gene. *J. Gen. Virol.*, 83(1):141-50, 2002.

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