

# Human anelloviruses: an update of molecular, epidemiological and clinical aspects

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**Abstract** Human torque teno viruses (TTVs) are new, emerging infectious agents, recently assigned to the family *Anelloviridae*. The first representative of the genus, torque teno virus (TTV), was discovered in 1997, followed by torque teno mini virus (TTMV) in 2000, and torque teno midi virus (TTMDV) in 2007. These viruses are characterized by an extremely high prevalence, with relatively uniform distribution worldwide and a high level of genomic heterogeneity, as well as an apparent pan-tropism at the host level. Although these viruses have a very high prevalence in the general population across the globe, neither their interaction with their hosts nor their direct involvement in the etiology of specific diseases are fully understood. Since their discovery, human anelloviruses, and especially TTV, have been suggested to be associated with various diseases, such as hepatitis, respiratory diseases, cancer, hematological and autoimmune disorders, with few arguments for their direct involvement. Recent studies have started to reveal interactions between TTVs and the host's immune system, leading to new hypotheses

for potential pathological mechanisms of these viruses. In this review article, we discuss the most important aspects and current status of human TTVs in order to guide future studies.

## Discovery of torque teno viruses

The torque teno viruses currently known to infect humans include three closely related groups of strains. They were discovered starting in 1997 and have recently been assigned to three genera in the family *Anelloviridae*: torque teno virus, genus *Alphatorquevirus*; torque teno mini virus, genus *Betatorquevirus*; torque teno midi virus, genus *Gammatorquevirus* [1]. The family *Anelloviridae* also includes viruses that infect animals, such as pigs (torque teno sus virus, TTsuV), cats (torque teno felis virus), dogs (torque teno canis virus) and tupaia (torque teno tupaia virus) [76]. Anellovirus sequences are continually being described in animals; the most recently discovered sequences were found in UK rodents [131], *Tadarida brasiliensis* bats [30], and humans [117, 147].

Ever since the discovery of torque teno virus, new viral isolates have been described and identified at a high rate. A brief history of their discovery is presented in Figure 1.

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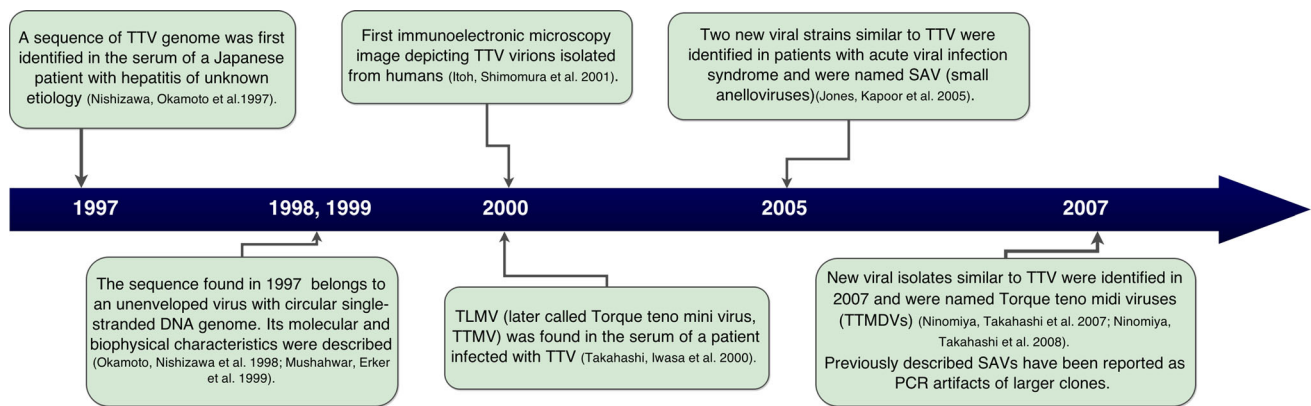
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## Structure and genome

### Structure

TTVs are unenveloped viruses with a capsid (composed of 12 pentameric capsomers) that has  $T = 1$  icosahedral symmetry ([http://viralzone.expasy.org/all\\_by\\_species/772](http://viralzone.expasy.org/all_by_species/772)).



**Fig. 1** Timeline of human anelloviruses discovery landmarks. Works cited in the diagram are in the order they appear: [82, 85, 124, 128, 129, 132, 137, 174]

html), and the virion has a buoyant density of 1.31–1.34 g/cm<sup>3</sup>. Filtration studies have shown that the virion particle size ranges between 30 and 50 nm for TTV [124] and is less than 30 nm for TTMV [173].

TTVs cannot be propagated *in vitro* due to the lack of compatible cell systems, and therefore there have been few studies in which their structure and function have been investigated in a direct manner. The only paper showing an electron microscopy image of TTV virions was published by Itoh *et al.* [81]. The virions were from sera and fecal supernatant of HIV-infected individuals. Immunogold electron microscopy revealed that they were bound to human immunoglobulin G. However, up to now, no structural studies (*e.g.*, X-ray crystallography) of anelloviruses have been conducted.

## Genome

The best-studied component of TTVs, their genome, consists of a circular single-stranded DNA molecule of negative polarity, with a GC-rich region of 117 nucleotides (89%–90.6%). Genome size varies between different isolates, ranging from 3.6 to 3.9 kb for TTV [63, 119, 148], 3.24 to 3.25 kb for TTMDV [127], and 2.86 to 2.91 kb for TTMV [142] (Fig. 2).

The TTV genome can be divided into an untranslated region (UTR) of 1.2 kb and a potential coding region of 2.6 kb. The UTR region is relatively conserved, suggesting that it plays an important regulatory role in viral replication. The coding region of anelloviruses contains two large open reading frames: ORF1, encoding a protein of 770 amino acid residues for TTV (isolate TA278 sequence), 637 amino acid residues for TTMDV (isolate MD1-073 sequence), and 663 amino acid residues for TTMV (isolate CBD231 sequence); and ORF2, encoding of protein of 120, 123 and 91 amino acid residues for TTV (isolate TA278 sequence), TTMDV (isolate MD1-073 sequence) and

TTMV (isolate CBD231 sequence). Several other open reading frames have been described, and the peptides that they encode differ in length for different isolates.

TTV isolates are estimated to have 47–70% divergence in the amino acid sequences of their encoded peptides [11, 99]. These differences are not evenly distributed throughout the genome; most of the variability occurs in the coding region, which has three hypervariable regions [133], while the untranslated regions are less variable than the regulatory sequences [94].

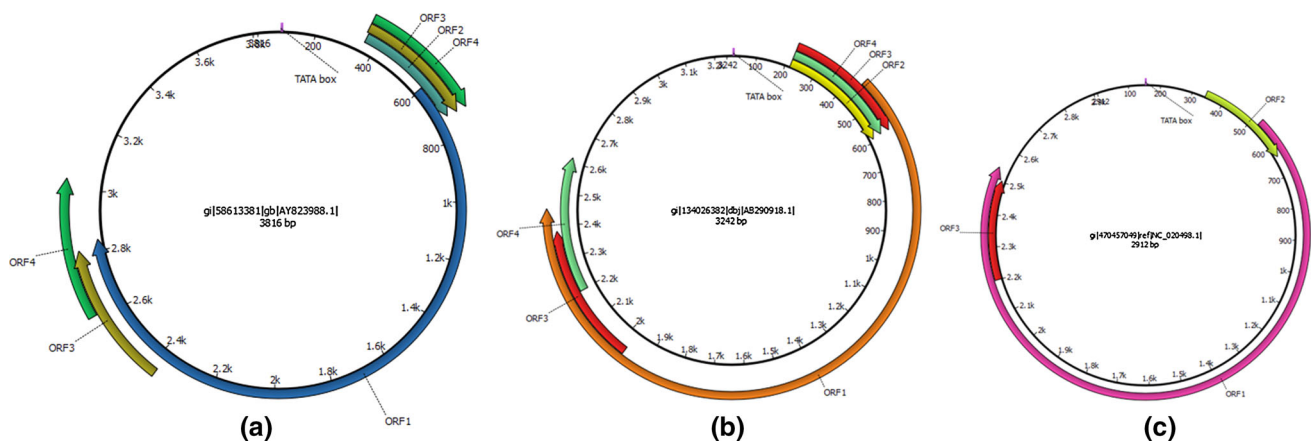
## Genome sequence heterogeneity

In the early 2000s, due to the very high genetic variability of these viruses, TTV were classified into five genetic groups with at least 50% nucleotide sequence divergence. Each group contains a number of genotypes with differences of up to 30% in terms of genomic sequence [13, 63, 64, 142]. Several different genotypes can co-infect the same individual at the same time, and different combinations of viral genotypes often predominate in different tissues [9].

Phylogenetic analysis has shown that TTVs form a large phylogenetic tree with five major clusters [13]. The reason for the extensive genomic variation and the mechanism by which it occurs is not known, but several hypotheses have been proposed.

One hypothesis is that TTVs mutate at a very high rate. There are many studies in which the nucleotide sequences from multiple isolates were compared, showing either the persistence of the same sequence throughout the years or alterations of the sequence in the host [9, 11, 48, 78].

The high mutation rate is uncharacteristic of DNA viruses, because they lack their own replicative machinery and therefore use the host's DNA polymerases, which have a high level of proofreading ability. However, it has been shown that the ssDNA genomes of parvoviruses evolve at



**Fig. 2** Schematic illustration of the genome organization of torque teno viruses. a) TTV isolate 2h (GenBank accession no. AY823988), b) TTMDV isolate MD1-073 (GenBank accession no. AB290918), c)

TTMV isolate TTMV\_LY1 (GenBank accession no. NC\_020498.1). The arrows represent open reading frames. Annotations and illustrations were made using UniPro UGENE software version 1.11.4 [145]

high rates, and it has been suggested that this increased rate of mutation could be explained by the nature of the single-stranded genome and its capacity to encode proteins involved in replication [167]. It is thus possible for TTV to have a mechanism that allows it to accumulate mutations at a high rate.

The majority of amino acid substitutions in TTV isolates were located in sequences encoded by hypervariable regions of ORF1. The hypervariable regions of the TTV genome may represent mechanisms of immune response avoidance and chronic infection [83].

Another hypothesis suggests that only a small part of TTVs have all the necessary components to successfully infect hosts, or, in other cases, co-infections with multiple isolates may bring together the components needed for infection [91, 99]. Furthermore, a very recent study has demonstrated that a herpesvirus, Epstein-Barr virus, acts as a helper for TTV [17].

Another mechanism by which viruses evolve is recombination, and for TTV, recombination events have been estimated to occur frequently [111, 194]. However, Jelcic *et al.* [83] have disputed this, arguing that the recombination events that have been reported are artifacts resulting from PCR.

More recently, the intra-genomic recombination has been suggested as a cause of the heterogeneity of the TTV genome [95]. Additionally, frequent recombination phenomena may mask the differences between viral populations [111]. Regarding TTV, it has been suggested that the virus co-evolved with the host over millions of years, resulting in enormous variability [9, 11, 48, 64, 156]. Nonetheless, whatever the mechanism behind TTV heterogeneity, it has become clear that there are no completely monotypic infections, but rather infections with a broad group of viruses [87].

### Genome replication

TTV has a high *in vivo* replication capacity. The kinetics of viral load and clearance during interferon therapy have suggested that more than  $10^{10}$  TTV virions are produced every day, while 90 % of the virus in the plasma is removed and replaced during this period [103].

TTV genome replication seems to be controlled by the immune system (higher viral titers were found in individuals with immunodeficiencies, such as AIDS [168, 183] and other intercurrent diseases [201]).

Concerning the tropism and cell-specificity of TTV, studies have shown that TTV does not have a particular tropism, and viral genome replication can occur in several tissues/organs (*e.g.*, liver [141], bone marrow [92], lymph nodes, spleen, pancreas, thyroid, muscles, lungs, kidneys, and PMBCs [102, 138, 143, 154]). Nevertheless, viral loads were up to 10–300 times higher in certain tissues (*e.g.*, bone marrow, lung, spleen, liver) than in the serum of the same individual [137]. It was also observed that the TTV genotypes found in plasma and PMBCs often differ [138], which could represent a compartmentalization of the TTV infection similar to that observed with other viruses (*e.g.*, HIV-1, HCV) [100].

The exact mechanism of TTV replication is not fully understood. Based on similarities to other ssDNA-genome viruses, it is assumed that TTV uses the rolling-circle replication mechanism [124]. ORF1 has been shown to encode a protein that contains Rep motifs [43, 99, 124, 181].

The genomic diversity of TTV has also led to the hypothesis that viral DNA is replicated by a machinery with poor or absent proofreading activity. Another hypothesis is that TTV might replicate through an RNA intermediate, like hepatitis B virus, although it seems unlikely because

the TTV genome does not encode a reverse transcriptase [133].

### Phylogeny

Until recently, TTV formed five major phylogenetic clusters, including tens of viruses with over 35 % nucleotide sequence divergence in ORF1 [16]. Genogroup 3 is believed to contain the largest number of TTV isolates described to date, while genogroups 2 and 4 are less prevalent than the others [14, 41, 60, 104, 151].

Likewise, TTMV is highly divergent; the first phylogenetic tree based on TTMV genomic sequences revealed a large cluster of strains that diverge from each other by over 42 % in ORF1 at the nucleotide level and over 67 % at the amino acid level [15, 173].

Data about TTMDV are scarce; however, recent databases inquiries have shown several complete genomic sequences.

Currently, ICTV classification is based on ORF1 analysis in its entirety, with a cutoff value of 35 % nucleotide sequence identity being the species demarcation criterion for the three anelloviral genera (*Alphatorquevirus*, *Betatorquevirus* and *Gammatorquevirus*). *Alphatorquevirus* includes 29 species (*Torque teno virus 1–29*), while *Betatorquevirus*, which includes 12 species (*Torque teno mini virus 1–12*), and *Gammatorquevirus*, which includes 15 species (*Torque teno midi virus 1–15*), include other related viruses that may be members of these genera but have not been assigned to separate species [1].

To better illustrate the diversity of human anelloviruses, we selected complete ORF1 sequences from GenBank and proceeded to construct the phylogenetic tree (Fig. 3). The tree was built using MEGA 6 software with the UPGMA algorithm, the Jukes-Cantor substitution model and 1000 bootstrap replicates [177]. Tree annotation was performed using EvolView [197]. The name of the isolates used for the phylogenetic analysis along with the accession number and references are found in Online Resource 1.

### Prevalence of torque teno viruses

#### *TTV, TTMV and TTMDV prevalence in serum and/or blood*

The reported prevalence of TTV in blood has varied greatly in recent decades, both within and between different populations around the globe.

Initially it was estimated that the prevalence of TTV is low. Subsequently, information about the heterogeneity of the TTV genome arose, and various PCR methods using different combinations of primers that can detect, quantify and/or differentiate TTV genotypes and related viruses were developed [12, 41, 69, 106, 120, 129].

The rate of TTV-positive sera reported for the general population in the last 5 years varies between 5 % in Brazil [34] and greater than 90 % in Russia, Japan and Pakistan [73, 129, 191]. Despite the fact that the detection rates fluctuate greatly, it is estimated that over 50 % of the general population was infected with TTV in the early 2000s [48, 59, 67].

The highest TTV prevalence has been found in poly-transfused thalassemic patients, long-term hemodialysis patients, hemophiliacs treated with clotting factor, and intravenous drug users. The high TTV titer may reflect the immune status of the individual; immunodepressed patients have high viral DNA titers [183]. Furthermore, monitoring TTV titers in patients undergoing hematopoietic stem cell transplantation has been used to evaluate immune recovery [45, 185].

The shift in the prevalence values reported in the literature over time was due to a large extent to the methods used for the detection of the viral genome. After the discovery of TTV, its detection was done by PCR with primers targeting ORF1 (N22 region, the first described sequence). As UTR regions of the viral genome are more conserved when compared to the ORF regions, UTR-targeting primers (used later for the detection of TTV DNA) detect a larger number of genotypes [65] and thus have a higher overall detection rate.

Regardless of the shortcomings of various techniques, it has become clearer that TTV has a high prevalence in the general population worldwide (Online Resource 2) and that prevalence increases with age [67, 162, 187, 199]. Figure 4 shows a comparative view of TTV prevalence in humans when assessed by amplifying the non-coding vs. the coding region of the TTV genome.

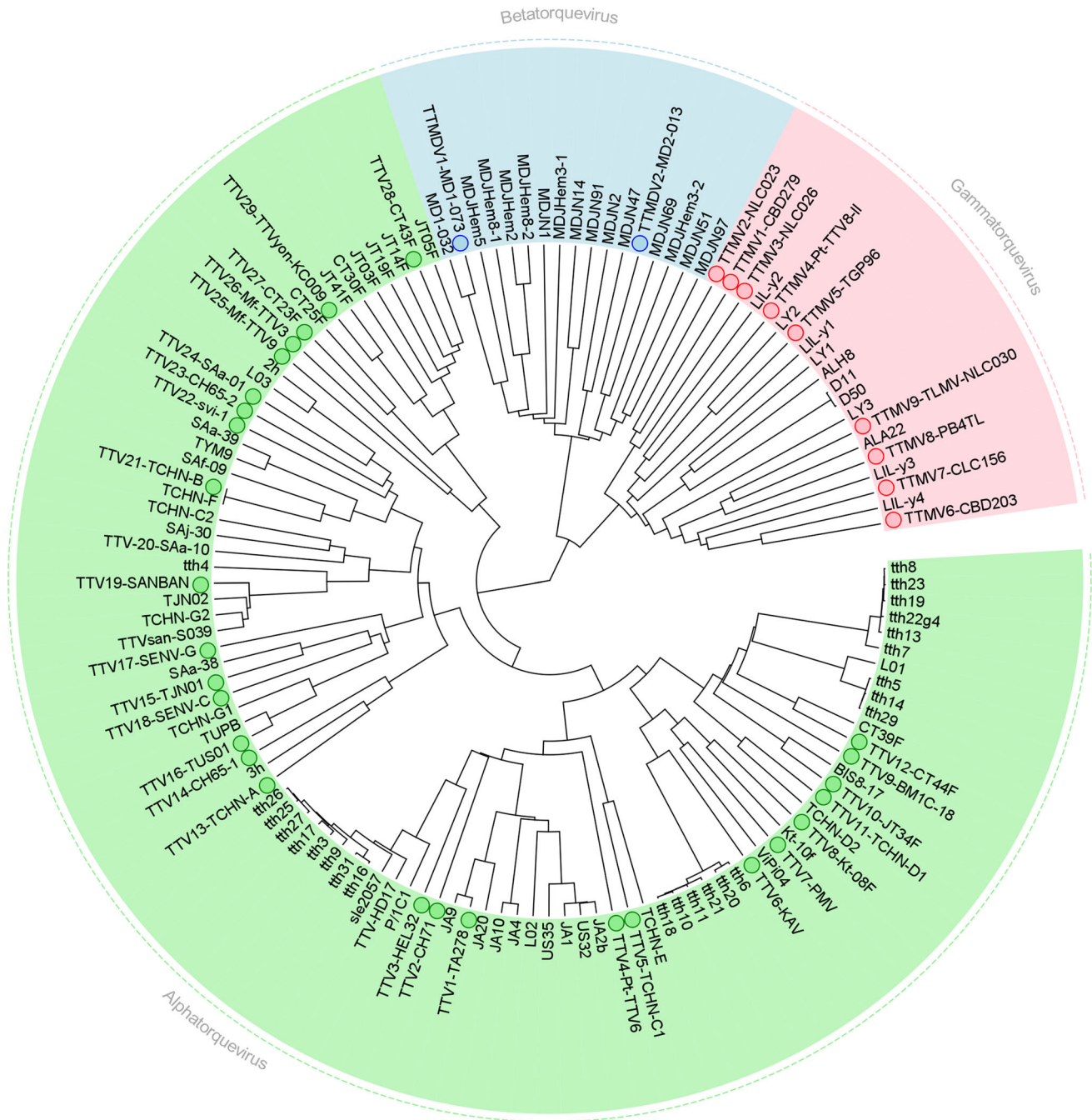
It has been proposed that TTV infection has a different geographical distribution and that some genotypes emerged in isolated ethnic groups [156, 188, 190]; however, several studies have disputed these hypotheses [49, 71, 111, 124, 152].

Studies from the mid 2000s showed that some viral genogroups are more common than others [107, 109], although this may be attributed to the use of certain genogroup-specific primers or even to some peculiarities of the viral genotypes. Moreover, prevalence and viral titer variations can be explained by the use of slightly suboptimal PCR conditions [71].

The other recently discovered human anelloviruses (TTMV and TTMDV) have been studied less, and reports on their prevalence are fewer. The prevalence of TTMV ranges from 48 % in Norway [121] to 80.15 % in Japan [129], whereas TTMDV is present in at least 40 % of the general population [127].

The new detection methods that have been developed are leading to the discovery of new viral variants [15, 126]. The





**Fig. 3** Phylogenetic tree of human anelloviruses. The tree was built with MEGA 6 software [177], the UPGMA algorithm, the Jukes-Cantor substitution model and 1000 bootstrap replicates and annotated with EvolView [197]. Available complete ORF1 sequences of

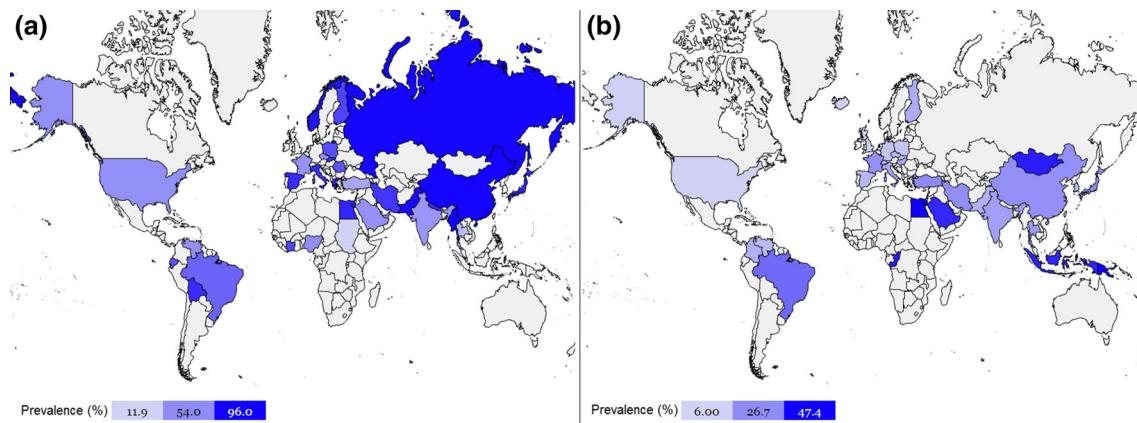
real situation of TTVs prevalence in humans is an important topic for future studies. However, it is clear that the majority of healthy individuals have the virus present in their blood.

#### *TTV prevalence in other biological samples*

Using PCR-based methods, TTV DNA has been detected in different organs, tissues and biological samples, including

TTV, TTMV and TTMDV were downloaded from GenBank (Online Resource 1). The reference isolate for each viral species is indicated by a circle

saliva [38, 49, 75, 115, 161], urine [115, 161], perspiration [115], tears [115], nasal secretion [106], feces [49, 115, 136, 161], throat swabs [79], liver [84, 97, 153, 159], bile [186], gastric tissue [105], cervical secretion [23, 46], semen [75, 113, 115], hair and skin [146], bone marrow [141], lymph nodes, muscles, umbilical cord blood [116], thyroid, lungs, spleen, pancreas [141], kidneys, cerebrospinal fluid [101, 153, 169], nervous tissue [169] and PMBCs [143, 196].



**Fig. 4** Comparative world heat maps of TTV prevalence in humans, detected using UTR- (a) vs. ORF- (b) derived primers. Data (percentages) represent the average prevalence for each country in

Online Resource 2, regardless of the number of entries or year. Grey areas represent countries with no data

In blood, TTV does not have specificity for a given cell type and has been identified in B and T lymphocytes, monocytes, granulocytes and NK cells, but not in red blood cells or platelets [102, 175]. However, there are conflicting views about the presence of viral DNA in B [51] and T lymphocytes [196].

In addition to TTV DNA, its mRNA has been identified in PMBCs, cerebrospinal fluid, breast tissue, colon, bladder, liver, bone marrow and thyroid [112, 139, 153].

TTV DNA has also been detected in tumor tissues [36], including brain tumors [169]. Nonetheless, as noted by Takahashi *et al.* [175], since TTV is present in blood and immune cells, it is difficult to accurately determine whether the viral DNA detected was derived from the actual tissue cells analyzed or immune cells from the blood flow.

#### *Prevalence of TT viruses in non-biological sources*

Given its global ubiquity, fecal-oral route of transmission, lack of seasonal variation, and structural similarity (size and composition) to enteric viruses, TTV is used as an indicator of viral contamination [57].

In Manaus County of the Brazilian Amazon, more than 90 % of the 1.7 million inhabitants are deprived of wastewater collection systems, and thus waste is spilled into the Negro River. A team of researchers collected 52 water samples four times (August, November, February and June) from 13 locations in the region for a period of one year. TTV was detected in 92.3 % of the samples of surface water, ranging from 1,300 to 746,000 TTV genomes in 100 ml of water. The presence of TTV DNA did not fluctuate according to season and geographical area, and the TTV load did not correlate with the abundance of coliform bacteria or physicochemical parameters [42].

Nevertheless, the prevalence of TTV was similar to the prevalence of HCV reported in 2007 in the same geographical area [35].

In Europe, to assess the rate of TTV positivity in Italian waters, Veran *et al.* collected samples of treated river water monthly for one year reporting that TTV was present in 3 out of 12 samples (25 %) [192].

Studies in Japan showed that TTV DNA was present in 5 % of the surface water in that country, without seasonal variation [61]. When TTV was monitored in eight active sludge-treatment plants in Japan every month for 1 year, researchers reported that TTV genetic material was detected with a frequency of 97 % in the influent and 18 % in secondary effluent after treatment with activated sludge before chlorination, 24 % in the final effluent after chlorination and 0 % in the effluent for reuse after filtration and ozonation [62]. They also concluded that chlorination does not affect PCR detection of TTV genetic material, although it can inactivate the virus without affecting the genomic regions amplified [134].

In India, Vaidya *et al.* [189] compared the titers of hepatitis A and E viruses and TTV in influents and effluents of wastewater treatment plants. They observed that the prevalence of TTV DNA in untreated wastewater (12.7 %) was statistically similar to the prevalence of hepatitis E virus RNA (11.0 %) and hepatitis A virus RNA (24.4 %). In effluent water, unlike hepatitis A virus, TTV and hepatitis E virus did not show statistically significant decreases.

TTV DNA was also detected on 14.9 % of hospital surfaces tested by Carducci *et al.* [21] and on 31 % of surfaces, as well as 16 % of air samples contaminated through toilet use [193]. The authors suggested that TTV could be used as an indicator of general viral contamination of the environment.

## Transmission routes

The main route of transmission of TTVs is believed to be by blood, as it is commonly present in people exposed to infected blood and blood products (e.g., transfusions or hemodialysis) [2, 77, 114, 157], or in people using intravenous drugs [6, 7]. Even so, parenteral transmission (e.g., by injection) does not explain the global prevalence and ubiquity of TTV viruses. Viruses are transmitted efficiently through saliva, and TTV is no exception; its titer in human saliva may exceed that in serum [38, 49]. The presence of TTV DNA in pharyngeal mucus [29] suggests that respiratory tract and salivary droplets are common dissemination paths. Moreover, a recent study has provided evidence of the presence of TTV in the breath of infected individuals [27]. It is possible that the main route of transmission would involve breath and saliva, as this would explain its high prevalence.

In infected individuals, TTV is also present in feces [98, 136], and TTV isolated from this source is able to infect susceptible and permissive cells *in vitro* [101]. Fecal-oral transmission of TTV is most likely achieved through progeny virions secreted in the bile, which are released later in feces [136, 186].

A secondary enteric transmission route of TTVs is by water. TTV has been identified in a number of aquatic ecosystems, being detected in 5 % of the surface water tested in Japan [61], but mostly in sewage water [62]. Griffin et al. [57] have proposed that international standards of water quality testing should include detection and quantification of TTV; current tests are based only on bacterial indicators, most often providing erroneous information on the presence of enteric viruses. Thus, TTV can become an effective indicator for risk assessment for viral pathogens in water.

TT viruses have recently been detected in food, supporting the oral route of transmission. TTV DNA was found in raw and pasteurized camel milk from the United Arab Emirates [3] and in raw, but not pasteurized, buffalo milk and dairy products from Italy [160]. TTV and TTMV DNA were also detected in meat and meat products collected from local supermarkets in Romania [170].

Given the high rates of infection with TTVs, alternative routes of transmission have been considered, including transplacental transmission, transmission through the umbilical cord blood [123, 162], or contact with saliva, skin, or hair of infected individuals [146].

Sexual transmission is another possible route of transmission of TTVs in the human adult population, considering that they have been detected in the cervical epithelium and in semen [19, 23, 44, 75, 113]; recent studies have shown that TTV has the ability to multiply with high frequency in the cervical epithelium [198].

However, some researchers believe that sexual transmission is of minor importance for TTV given the fact that the infection may occur early in life or even *in utero*, and various TTV genotypes have been detected in newborns, umbilical cord blood and amniotic fluid [8, 55, 116, 123, 162]. Other findings contradict the hypothesis of intrauterine transmission of TTV, claiming that healthy newborns may come in contact with the virus a few months after birth from the environment [33, 90, 129] or breast milk [55, 80, 116, 135, 165]. The conflicting data obtained may be the consequence of the PCR methods used for viral DNA detection [116] and the low viral load in children.

TTV sequence comparison from isolates identified in children and their mothers have shown both similarity and divergence, confirming that TTV is acquired by children in more than one way – from the mother and from the environment [8, 33, 96, 135, 171].

As for the newly discovered TTVs, TTMV has been detected in umbilical cord blood, breast milk, amniotic fluid, and serum of newborns, and these viruses show sequence similarity to those of the mother [116]. The mother-child transmission route may explain the apparently ubiquitous and persistent infections.

## Involvement in human pathology

TTVs are characterized by a high prevalence in the general population. The possible involvement of these viruses in human pathologies has been debated ever since their discovery. Certainly, a nearly ubiquitous virus is unlikely to be pathogenic *per se*. Although TTV, TTMDV and TTMV are potentially associated with many diseases, there are conflicting opinions about their disease-causing potential in the human populations due to their ubiquity. It is possible that only certain genotypes/genogroups/species of human anelloviruses may be particularly pathogenic.

While TTV was originally believed to be a new viral etiologic agent of hepatitis [132], following the description of the virus' prevalence in most of the general population of the globe, researchers have tried to associate it with a specific or non-specific pathology. In 1999, Griffiths *et al.* [58] introduced the concept of a commensal virus to describe the TTV-host relationships.

Those who are convinced of the lack of TTV pathogenicity believe that this could be the result of long periods of virus-host co-evolution or the lack of specific cell ligands. Nevertheless, epidemiological studies have provided evidence about the association of TTV with several pathological conditions of the liver, blood, immune system, respiratory system, cancers, etc. However, these studies do not paint an accurate and complete picture of the involvement of TTV in human pathology [54].

In the last decade, only a few studies have started to scratch the surface of the presumable pathogenic potential of TTV and the mechanisms behind it. The expression of genotype 1 TTV ORF1 in transgenic mice produces a protein that causes pathological changes in the kidney. This protein appears to interfere with renal epithelial cell differentiation [195]. A recent study has shown that certain TTV isolates are activators of pro-inflammatory cytokine production through Toll-like receptor 9 (TLR-9) and thus may increase the severity of inflammatory diseases [158]. Moreover, Kincaid *et al.* [93] showed that TTV encodes an miRNA *in vivo* that targets N-myc (and STAT) interactor (NMI), thus mediating a decreased response to IFN and increased cellular proliferation in the presence of IFN. These facts support the theory that miRNA-mediated immune evasion contributes to the immense ubiquity of these viruses.

The pathogenic mechanisms of TTVs remain cryptic and still constitute the *tabula rasa* of TTVs research.

## Hepatitis

TTV has been discovered in a case of post-transfusion hepatitis. Furthermore, it was shown that it can multiply in hepatocytes both *in vivo* [140] and *in vitro* [39], and therefore numerous subsequent studies were focused on the involvement of TTV in liver pathology.

Okamoto *et al.* reported TTV titers 10 to 100-fold higher in liver tissue than in serum from patients with transfusion-acquired hepatitis [137]. They also noted that in recently infected patients, alanine transaminase (ALT) levels increased with viral titer, with TTV becoming undetectable in patients with normalized ALT levels [132].

Given the high rate of asymptomatic infection with TTV in the general population, it is most likely that hepatitis is not caused by TTV infection *per se* [5, 130]. However, TTV has been associated with fulminant hepatitis [24, 25, 72, 125]. Tanaka [179] and Desai *et al.* [40] reported that abnormal liver profiles were common among viremic individuals. A previous study that focused on highlighting the cytopathic effects of TTV in hepatocytes showed that liver function in most cases of hepatitis with TTV is abnormal; liver tissue appears discolored, cells are swollen, and acidophil degeneration, formation of apoptotic bodies and focused necrosis develop. However, inflammation of the lobules and portal system is mild [70].

Another series of studies reported possible associations between TTV infection and liver pathology [24, 74, 88, 144, 178, 184] such as recurrent acute hepatitis (genotype 13 TTV), reinforcing the hypothesis that TTV may be involved in mild forms of liver disease [47]. It has also been suggested that persistent TTV infections can contribute to cryptogenic liver failure in hemophiliacs [176].

A further assumption made about the involvement of TTV in liver pathology is that certain viral genotypes have a higher pathogenic potential than others, or even that some particular genotypes are associated with hepatitis. Genotype 1 has been incriminated in the occurrence of post-transfusion hepatitis [180], and genotype 1a has been reported predominantly in children with fulminant hepatitis and chronic hepatitis [144].

## Clinical significance of co-infections with TTV and hepatitis viruses

Concurrent infections with TTV and hepatitis viruses (HBV and HCV) have been extensively studied. High TTV viral loads have been associated with the development of hepatocellular carcinoma in patients with chronic hepatitis C [182]; moreover, the histological grade appears to be higher in hepatocellular patients with TTV-HCV co-infection than in those infected with only HCV [164]. Moreover, it has been suggested that ORF3 of TTV genotype 1a encodes a protein similar to the non-structural protein 5A (NS5A) encoded by HCV. This protein plays a major role in the IFN-induced antiviral response [182].

The mortality rate in patients with HBV-TTV co-infection is higher than in patients with HBV infection alone [40]. However, no clear evidence has been provided for the involvement of TTV as a causative agent, co-factor or marker for the occurrence or progression of liver disease.

Another series of studies has shown that suprainfection of HBV or HCV patients with TTV does not seem to have any effect on the severity of disease manifestations [20, 68, 89, 166, 184]. Although TTV is frequently detected in patients chronically infected with HBV and HCV, it does not seem to have any influence on the histopathological characteristics of the HBV- or HCV-infected liver. Chattopadhyay *et al.* have shown that the clinical and biochemical profiles of patients with chronic hepatitis or HBV and HCV co-infection with TTV were not significantly different from those without TTV [26]. However, the possibility that TTV contributes to the progression of liver disease in people infected with HBV or HCV cannot be totally excluded [40, 122].

Data on the relationship between TTV and liver diseases have been found to be inconsistent, since there is an extensive range of evidence indicating the absence of any such correlation. The lack of morphological changes in hepatocytes that are positive for TTV DNA by *in situ* hybridization casts doubt on a role for TTV in liver pathogenesis.

Kadayifci *et al.* [86] compared the rate of detection of viral DNA in patients with elevated ALT and healthy people and did not find any significant difference. Histological examination also did not reveal any visible



cytopathic effect that could be attributed to viral infection with TTV.

Once again, the conflicting data leave the matter of TTV involvement in liver diseases unresolved.

### Respiratory diseases

Lung tissue has been shown to be one of the sites where TTV can multiply [10]. TTV infection has been suggested to play a role (either as an active or opportunistic pathogen) in children with acute respiratory disease. Higher TTV viral loads were found in nasal swabs from patients with severe respiratory diseases such as bronchopneumonia [106]. Also, TTMDV prevalence appears to be higher in children with acute respiratory distress syndrome [29]. Furthermore, children with increased viral titer in nasal specimens were found to have weak spirometric values. It also appears that TTV may contribute to the pathogenesis of asthma; it has been hypothesized that TTV replication could direct the immune response toward the Th2 pathway, which is known to be involved in asthma pathogenesis [149].

In patients with idiopathic pulmonary fibrosis, TTV infections are associated with the severity of bronchiectasis, and even with a low rate of survival [150]. Also, a TTMV isolate, namely TTMV-LY, was identified in pleural effusion and may potentially be associated with severe pneumonia in children. The authors found that TTMV-LY can deeply colonize the lungs; alveolar epithelial cells are permissive for TTMV-LY. The infection modulates the innate immune response in lung cells by inducing the expression of pro-inflammatory cytokines (IFN- $\gamma$ , CCL5, IL-2 and IL-12) [50].

However, as in the case of the other diseases mentioned above, it is not clear whether TTV infections are the cause or the result of disease progression.

### Cancer

Consistent with the ubiquity of human anelloviruses, TTV DNA has been found in a variety of neoplastic tissues [36]. However, this could be supported by tissue inflammation or rapid division of cancer cells that support the replication of the TTV genome.

zur Hausen *et al.* have put forward the interesting hypothesis that persistent infections with TTV increase the risk of specific translocations, thus having an effect on the development of leukemia and lymphoma in children [202]. Moreover, TTV genotype 10 was identified in a patient with chronic lymphocytic leukemia and polycythemia vera [28]. Although TTV DNA was identified in various types of lymphoma with the same prevalence as in healthy

individuals and the virus was found in non-neoplastic cells, some researchers have postulated that TTV may modulate infected T lymphocytes and thus play a role in the pathogenesis of lymphomas [51].

Co-infection with human papillomavirus (HPV) and TTV genotype 1 has been associated with poor prognosis in patients with laryngeal cancer, but it is unknown whether TTV has any effect on cancer progression [172].

TTV DNA has been detected with a significantly higher frequency in patients with sporadic breast carcinoma in association with the eNOS bb (eNOS 4a/5b VNTR) genotype or the presence of the ACE I (ACE ID VNTR) allele compared to healthy women [31].

The presence of TTV in colorectal carcinoma specimens may be associated with pathogenesis, but the same TTV isolates have also been detected in normal tissue [37].

TTV DNA is frequently detected in sera from patients with Kaposi's sarcoma. Furthermore, TTV DNA is detected in the epithelial cells of the lesions and normal skin of these patients, but not from healthy subjects. It has been suggested that TTV infection might have an effect on the replication of the human herpesvirus 8, and thus a role in the pathogenesis of the disease [56].

TTV has been identified at a tenfold higher rate in PMBCs of patients with various types of cancer than in healthy subjects. However, it is unclear whether this is a feature of neoplastic pathology or if it is common in patients with severe diseases in general [200].

### Hematological disorders

Hepatitis-associated aplastic anemia occurs in particular after non-A, non-B, non-C acute hepatitis [18]. High levels of TTV in the bone marrow have been suggested to be responsible for aplastic anemia of unknown etiology [92, 118]. This possible association, however, was not supported in other studies [155, 163].

TTV has been found with high prevalence, ranging between 50.5 % [4] and 100 % [22], in patients with thalassemia. Studies have also shown that thalassemic patients were positive for TTV DNA in different biological samples (*e.g.*, PMBC, plasma, saliva, urine) with the highest rate of prevalence in PMBCs and plasma (greater than 90 %), and the lowest prevalence in urine (22 %). Also, more than 45 % of the patients had co-infections, and the most frequently found genogroup of TTV was genogroup 2 [22].

Regarding the association of TTV with hematopoietic malignancies, TTV DNA was detected in lymphocytes and lymph nodes from patients with B-cell and Hodgkin's lymphoma [51]. It has been postulated that TTV may modulate the functions of infected T cells and thus may play a role in the pathogenesis of lymphoma.

## Autoimmune disorders

The etiological agents for many autoimmune diseases are not yet known, and investigations have been carried out, taking into consideration the subtle interplay between TTV and the immune system. Costa *et al.* reported the presence of TTV and even co-infection with multiple TTV genotypes in patients with systemic lupus erythematosus (SLE). Although this study revealed an association between TTV infection and SLE, the number of TTV genotypes infecting the patients did not appear to have an effect on SLE phenotype [32].

Gergely *et al.* observed that peptides encoded by TTV ORF1 and ORF2 show similarity to a human endogenous retrovirus-encoded nuclear protein (HRES-1/p28), which generates antibody epitopes common in cases of SLE. In addition to the high titers of TTV, patients also had high titers of anti-ORF1 and anti-ORF2 antibodies. However, it is unclear whether TTV plays a role in the production of these auto-antibodies, or if, due to immunological causes, patients may be more prone to infections [53].

TTV has been found in patients with rheumatoid arthritis [53, 66], idiopathic inflammatory myopathy [52], and multiple sclerosis (MS) [169]. Sospedra *et al.* have shown that T cells derived from MS patients reacted against arginine-rich peptide regions that were similar to the amino termini of TTMV and TTV ORF1 peptides. They hypothesized that recurrent infection with TTV and other predisposing factors (genetic and/or environmental) lead to the expansion of these cells and have an impact on disease development [169].

Maggi *et al.* showed that patients with rheumatoid arthritis had higher titers of TTV than healthy subjects. The authors suggest that there may be a link between TTV and arthritis, but, as in the case of other diseases, a direct relationship remains in question [110].

A study using an animal model showed that the expression of TTV genotype 1 ORF1 in transgenic mice leads to a truncated protein that causes pathological changes in kidney cells. The expression of the protein appears to interfere with the differentiation of kidney epithelial cells [195].

Although TTV is potentially associated with several human diseases, its presence in the general population across the globe, the uncertainty of a virus-free negative control, and the lack of obvious cytopathic and cytotoxic effects on cells make opinions related to the pathogenicity of these viruses controversial and difficult to sustain. It has been suggested that small TTV sub-genomic fragments identified in human serum could play a role in diseases, as is the case with plant geminiviruses [95]. It is also possible that some genotypes/genogroups could be more pathogenic than others [106, 110], as in the case with HPV.

On the other hand, until it is definitively demonstrated that TTVs have a pathogenic nature, it must be taken into account that TTVs replicate in PMBCs and are inevitably found in infected or damaged tissues. Thus, the presence or load variation of TTV DNA in tissue samples cannot constitute a direct association between TTV and certain pathology. Thus, it is possible that the higher TTV titers observed in different patient groups are a secondary phenomenon due to the disease [14].

The pathogenic potential of TTVs is still unclear, although there are numerous studies that have investigated the relationship between them and different pathologies (*e.g.*, liver disease, acute respiratory disorders, AIDS development, various types of cancer, autoimmune disorders and kidney disease). TTV replication appears to be stimulated by vaccination with an antigen, suggesting that the viral titer may be enhanced due to co-infection with other pathogens [108]. TTV infection with multiple genotypes and low and fluctuating viremia are making the discovery of association with different pathologies extremely difficult. New PCR systems that enable the detection, differentiation and quantification of all genotypes and/or genogroups are desired.

## Conclusions

As TTV and its relatives appear to have infected primates and probably all mammals throughout their evolutionary history, an intriguing question is whether the absence of TTV infection would have any consequences, such as immune system dysregulation that may result from the absence of the substantial antigenic burden of TTVs in infected individuals. For all we know now, TTV-related viruses may have been key players in the vast process of evolution. These unresolved questions undoubtedly represent fertile areas for fundamental virology and immunology research in the future.

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