



UNIVERSITI PUTRA MALAYSIA

***DETECTION AND PHYLOGENETIC ANALYSIS OF TORQUE TENO VIRUS IN BLOOD SAMPLES OF HEALTHY INDIVIDUALS***

NUR SYAZWANI BINTI JARKASI

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**DETECTION AND PHYLOGENETIC ANALYSIS OF TORQUE TENO VIRUS  
IN BLOOD SAMPLES OF HEALTHY INDIVIDUALS**

NUR SYAZWANI BINTI JARKASI

Thesis Submitted to the School of Graduate Studies, Universiti  
Putra Malaysia, in Fulfilment of the Requirements for the Degree of  
**Master of Science**

**November 2019**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in  
fulfilment of the requirement for the degree of Master of Science

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BLOOD SAMPLES OF HEALTHY INDIVIDUALS**

By

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**November 2019**

**Chair : Zulkefley bin Othman, PhD**  
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Torque Teno Virus (TTV) is a single-stranded DNA virus which infects human population worldwide. TTV is characterized by exhibiting a vast genetic diversity generating a large number of variants that clustered into seven genetic lineages. High TTV prevalence was reported from several parts of Asian continent. However, there is a lack of information regarding TTV prevalence among healthy Malaysian population as well as its genetic diversity. In order to elucidate the prevalence of TTV in Malaysia, DNA extracted from 137 plasma samples of healthy individuals were screened for the presence of TTV DNA by PCR based on primers derived from different genomic regions (5' UTR, 3' UTR and N22). By using 5' UTR-derived primer, 70.80% of our studied population shows positive for TTV. For 3' UTR-derived primer, the result shows that 37.23% of the studied population positive for TTV. Meanwhile, 15.46% of TTV strains detected from 5' UTR PCR is positive for N22 PCR. Phylogenetic analysis based on 3' UTR region reveals that Malaysian isolates are clustered into four groups supported by high bootstrap value (89% to 100%). Pairwise genetic distance of 3' UTR region of Malaysian isolates shows small genetic distance range between 0.020 to 0.269 among them. Subsequently, one sample was selected for near full-length amplification. A total of 3349 bp TTV DNA was amplified, and the amplicon was cloned, sequenced and subjected to bioinformatic analysis. From the analysis, a new isolate of TTV, named TTVMY01, was identified. Phylogenetic analysis based on whole sequence and each region thereof revealed that TTVMY01 is clustered into genogroup 3a. From 29 TTV species, TTVMY01 exhibit smallest genetic distance with SANBAN species, a Japanese isolate. Several protein motifs identified in each predicted protein of SANBAN remain conserved in TTVMY01. With regards to 5' UTR, this study demonstrates high frequency of TTV viremia among healthy Malaysian population, and in accordance with the reports from other geographical regions. Identification of new TTV variant may provide additional information for its genetic diversity, and it should be taken into consideration for pathogenicity study in the future.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk Master Sains

**PENGESANAN DAN ANALISIS FILOGENETIK KE ATAS VIRUS TORQUE  
TENO DALAM SAMPEL DARAH INDIVIDU SIHAT**

Oleh

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*Torque Teno Virus (TTV) adalah virus DNA helai tunggal yang menjangkiti populasi manusia di seluruh dunia. TTV mempunyai kepelbagaiannya genetik yang menghasilkan sejumlah besar varian di mana ia diklasifikasi kepada tujuh kumpulan genetik. Kelaziman TTV yang tinggi dilaporkan di beberapa bahagian benua Asia. Walau bagaimanapun, kelaziman TTV serta maklumat genetiknya dalam kalangan penduduk Malaysia yang sihat masih kurang. Untuk menentukan kelaziman TTV di Malaysia, DNA telah diekstrak daripada 137 sampel plasma individu yang sihat dan TTV dikesan dengan menggunakan kaedah tindakbalas berangkai polymerase (PCR). Primer yang digunakan untuk penguatan DNA TTV diperolehi dari kawasan genomik yang berbeza, iaitu kawasan UTR 5', UTR 3' dan N22. Jujukan UTR 3' kemudian dianalisa untuk menentukan hubungan genetik antara mereka. Dengan menggunakan primer yang diperoleh daripada UTR 5', 70.80% daripada populasi kajian adalah positif TTV. Berdasarkan primer yang diperoleh daripada UTR 3' pula, keputusan menunjukkan bahawa 37.23% daripada populasi kajian adalah positif TTV. Sementara itu, 15.46% daripada strain TTV yang dikesan dengan menggunakan 5' UTR PCR adalah positif untuk N22 PCR. Analisis filogenetik berdasarkan jujukan 3' UTR menunjukkan bahawa pencilan Malaysia dikelompokkan kepada empat kumpulan yang disokong oleh nilai bootstrap yang tinggi (89% to 100%). Bandingan secara berpasangan untuk setiap jujukan 3' UTR menunjukkan perbezaan yang kecil antara 0.020 hingga 0.269 antara setiap jujukan tersebut. Seterusnya, satu sampel telah dipilih untuk mendapatkan hampir keseluruhan jujukan genom tersebut. Jujukan bersaiz 3349 bp telah diperoleh dan dianalisis menggunakan perisian bioinformatik. Daripada analisis tersebut, isolat baru telah dikenalpasti dan isolat ini dinamakan TTVMY01. Analisis filogenetik berdasarkan keseluruhan jujukan dan setiap bahagian daripadanya menunjukkan bahawa TTVMY01 tergolong dalam genogroup 3a. Daripada 29 spesis TTV, TTVMY01 mempunyai jarak genetik yang kecil dengan spesis SANBAN, pencilan Jepun. Berdasarkan 5' UTR, kajian ini menunjukkan bahawa*

kadar jangkitan TTV dalam kalangan penduduk Malaysia yang sihat adalah tinggi dan ia selaras dengan laporan dari beberapa kawasan geografi yang lain. Pengenalpastian varian TTV yang baru mungkin memberikan maklumat tambahan berkaitan kepelbagaian genetiknya, dan ia harus diambil kira untuk kajian berkaitan potensinya dalam pembentukan penyakit pada masa akan datang.



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## LIST OF ABBREVIATIONS

aa	amino acid
ALT	Alanine aminotransferase
BLAST	Basic Local Alignment Search Tool
bp	base pair
CAV	Chicken anaemia virus
dsDNA	double-stranded DNA
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCC	Hepatocellular carcinoma
HIV	Human immunodeficiency virus
HSC	Hematopoietic stem cell
HVR	Hypervariable region
ICTV	International Committee on Taxonomy of Viruses
mRNA	messenger RNA
NLS	Nuclear localization signal
ORF	Open reading frame
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
QV	Quality value
RCA	Rolling-circle amplification
RCR	Rolling-circle replication
RDA	Representational difference analysis
ssDNA	single-stranded DNA
TTV	Torque teno virus
TTMV	Torque teno mini virus
TTMDV	Torque teno midi virus
TAIP	TTV-derived apoptosis-inducing protein
UTR	Untranslated region
VP	Viral protein

# CHAPTER 1

## INTRODUCTION

### 1.1 Background of Study

Torque Teno Virus (TTV), classified into genus *Alphatorquevirus*, is a member of *Anelloviridae* family and occupied the most significant component of human virome (Focosi et al., 2016). TTV is characterized by having a vast genetic diversity with at least 29 species have been reported, exhibiting >35% sequence divergence based on nucleotide sequence of open reading frame 1 (ORF 1) (Biagini et al., 2012). Each TTV species consist of numerous strains that are clustered into seven major phylogenetic groups based on nucleotide sequences of entire ORF 1 (Biagini et al., 2012; Hsiao et al., 2016).

TTV is a small, non-enveloped virus made up of single-stranded DNA (ssDNA) (Okamoto et al., 1998b; Mushahwar et al., 1999). The circular structure of ~3.8 kb in length of TTV genome is divided into two main regions, namely coding (~2.6 kb) and untranslated region, UTR (~1.2 kb). Analysis on full-length genome of various TTV strains identified two conserved viral proteins, known as ORF 1 and ORF 2, within the coding region (Ukita et al., 2000; Muljono et al., 2001; Hussain et al., 2012). ORF 1, the longest viral protein, is predicted to encode a structural protein corresponding to the viral capsid. Meanwhile, ORF 2 is postulated to encode the non-structural protein, such as regulating the host's immune system during infection that contributes to TTV pathogenesis (Zheng et al., 2007). Several shorter ORFs, ORF 3 and ORF 4, are not conserved and it is encoded only by certain isolates (Okamoto et al., 2000; Muljono et al., 2001). ORF 3 is also a non-structural protein that has significant functional similarity to apoptin, an apoptosis-inducing agent of Chicken anaemia virus (CAV) (Kooistra et al., 2004), while the function of ORF 4 has yet to be ascertained. The UTR of TTV genome consists of GC-rich region and various elements responsible for transcriptional control (Miyata et al., 1999; Ukita et al., 2000; Kamada et al., 2004).

Plasma and serum samples of either healthy individuals or individuals with diseases have been widely used for epidemiological studies (Hussain et al., 2012; Abuodeh et al., 2015; El-Taher et al., 2015; Cancela et al., 2016). Other types of clinical samples have also been tested for the presence of TTV DNA such as feces, saliva and tumor biopsy (Brassard et al., 2015; Hettmann et al., 2016). Different TTV strains have been detected in over 80% of the general populations in several countries, and Asian populations are no exception. For example, Pakistanis (Hussain et al., 2012), Indonesian (Muljono et al., 2001) and Taiwan Indigenes (Hsiao et al., 2016) were shown to have high TTV viremia, whereby up to 90% of the population are infected. Results demonstrating low TTV prevalence among Asian healthy populations have also been reported in

India and Iran (Irshad et al., 2008; Doosti et al., 2011; Izadi et al., 2016). TTV has also been detected among individuals with disease. However, the involvement of TTV in the pathogenesis of disease is still a subject of scientific debate. In relation to hepatitis, there is a significant association between TTV and hepatitis B as reported by Al-Qahtani et al. (2016). In contrast, Hussain et al. (2012) reported that there is no significant association between TTV and hepatitis B and C. As reported by Brassard et al. (2015), comparison between diarrheic and non-diarrheic shows TTV was significantly higher among diarrheic people. Indeed, the viral load was significantly higher in stool of diarrheic ( $2.0 \times 10^7$  copies/g) compared to non-diarrheic ( $2.0 \times 10^3$  copies/g) (Brassard et al., 2015). Apart from that, detection of TTV genotype 10 in a patient with chronic lymphocytic leukemia and polycythemia vera suggesting its correlation with these disease (Chu et al., 2011). Although TTV-disease association is still controversy, there is a possibility that some TTV variant with rearranged genetic components may contribute to disease pathogenesis or it may serve as a co-virus with another infectious agent.

PCR is the most commonly used method for detection of viral DNA in clinical samples. The genomic region targeted for amplification by PCR account for variable rate in global TTV prevalence (Okamoto et al., 1999b). Extensive sequence divergence occurs at the coding region than in the UTR that translates to a greater disparity at amino acid level (Erker et al., 1999). Comparison between Kt-08F (Indonesian isolate) with prototype TA278, for example, shows that they share 58.7% of overall sequence homology. In brief, Kt-08F share 80.7% of sequence similarity with isolate TA278 at UTR, while only 53.1% of the coding region are identical (Muljono et al., 2001). Due to great genetic diversity of TTV, the selection of the genomic region subjected for amplification will give an impact on the prevalence of TTV. As previously reported, UTR-derived primers resulted in higher detection rates compared to ORF-derived primer (Muljono et al., 2001; Kalkan et al., 2005; Peng et al., 2015).

A person could be infected with TTV DNA at the early stage of life during pre- or postnatal (Schröter et al., 2000; Iso et al., 2001; Matsubara et al., 2001; Tyschik et al., 2018). However, the acquisition of TTV at the early stage of life is not necessarily via mother-to-child (Tyschik et al., 2017). Individuals who do not obtain TTV via vertical transmission may be infected via horizontal route of transmission (Davidson et al., 1999; Schröter et al., 2000). The horizontal route of transmission includes fecal-oral (Pinho-Nascimento et al., 2011; Brassard et al., 2015), air-borne (Maggi et al., 2003) and blood transfusion (Jalali et al., 2017; Hartono et al., 2018; Tyschik et al., 2018). This various route of TTV transmission might be responsible for the widespread of TTV infection among humans.

## 1.2 Problem Statement

Interest in investigating TTV has been stimulated by its high infection rate in the human population at several geographical locations. In Asia, high TTV infection rate has been reported among healthy individuals with no clinical manifestations. The Asian's regions presented numerous reports on TTV prevalence compared

to others, but at present no information regarding TTV prevalence in Malaysia has been reported. Besides that, several mechanisms such as host-viral coevolution history, high rates of mutation, recombination event, and formation of sub-genomic self-replicating DNA (Shulman & Davidson, 2017), may resulted in large number of highly heterogeneous variants of TTV. Upon this, there is a risk that mutation on TTV will produce pathogenic variants that could significantly affect human health. Though TTV infections have not been causally associated with any specific disease yet; they may facilitate the progression of many diseases. By considering this, this study would like to address the following research questions:

1. What is the prevalence of TTV among healthy individuals in Malaysia?
2. How diverse is the Malaysian-isolated TTV compared to other known TTV isolates?
3. Which genogroup the TTV isolated from healthy Malaysian individual is categorized?

### **1.3 Significance of Study**

This study investigates the widespread of TTV among healthy Malaysian population and analysing the phylogenetic relatedness with the reported isolates. This study is essential as it provides the first data on TTV prevalence among healthy Malaysian population. Besides that, the existence of this virus poses a serious question on its pathogenic potential due to high level of sequence variability. The generation of large number of TTV variants may result from a high mutation rate. Mutations change the genetic materials of the virus and mutation that occurs may produce a pathogenic variant of TTV. Therefore, it is worthwhile to identify new TTV variants isolated from the uncharacterized region and analyse its whole genome in terms of the genomic organization, the coding region and its predicted protein, and sequence divergence against other strains of TTV. Understanding the sequence divergence exhibited by different TTV variants may provide valuable insight for *in vitro* study as well as for future outbreak.

### **1.4 Objectives**

The main objective of this study is to investigate TTV in blood of healthy individuals.

Specific objectives:

- i. To determine the prevalence of TTV isolated from blood of healthy individuals.
- ii. To determine the genetic diversity of Malaysian-isolated TTV based on its partial genomic sequence.
- iii. To characterize the near full-length genome of a selected Malaysian-isolated TTV.

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