Molecular and Cellular Probes xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Molecular and Cellular Probes

journal homepage: www.elsevier.com/locate/ymcpr

Development of a TaqMan-based real-time PCR assay for the rapid and specific detection of pigeon torque teno virus

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ARTICLE INFO

Keywords:
Pigeon torque teno virus
Real-time PCR
Vertical transmission

ABSTRACT

Pigeon torque teno virus (PTTV), a recently discovered circular DNA virus. Here, we developed a *Taq*Man-based real-time PCR for rapid and specific detection of PTTV infections with sensitivity up to 49.3 copies/μl. Positive signals can be observed by the assay in pigeon embryonated eggs, which indicted that PTTV can be transmitted vertically. Our findings play important implications for a better understanding the transmission of torque teno virus in pigeons.

Torque teno virus (TTV), also namely as transfusion transmitted virus, which was firstly found in plasma in Japan at the year of 1997 [1]. Since then, wide ranges of hosts were discovered with species-specific TTVs [2,3]. The TTVs were found in water sources, sewage and air, which means the viruses were environmental contaminants [4]. Previously research demosntated that different species-origin TTVs sharing various genomic characterization [5]. The TTVs genome lengths ranging from 2.1 kb (*Felis* species) to 3.9 kb (human) with more genetic diversity. However, TTVs were non-enveloped, single-stranded, negative-sense circular DNA viruses found in various species.

Torque teno virus was first identified from pigeon blood serum or pigeon disease samples in China in 2013 [6,7]. Then, pigeon torque teno virus (PTTV) was found and sequenced by us (FJ17290 strain, GenBank No. MF576435) in Fujian Province, Southeast China. Genomic analyses imply that PTTV shared closer molecular evolution relationship with chicken anemia virus (CAV) [7]. Pigeon breeding has a long history not in China but widely in worldwide, which have higher potential commercial value, especially with the rapid development of economic growth. Both healthy and diseased pigeons can be detected PTTV positive by conventional PCR, which need we pay more attention on the epidemiological and pathogenesis studies for the newly recognized virus [7]. Therefore, an adequate diagnostic technique for PTTV infection is extremely urgent. Here, a TaqMan-based quantitative real-time PCR (TaqMan qPCR) for PTTV infection was successfully developed. Best to our knowledge, it was the first time to describe TaqMan qPCR assay for PTTV.

PTTVs were used to bio-computational analyze in this study, including NJGC strain (GenBank No. KF477315), NJLH strain (GenBank No. KF477316), (NJJN strain (GenBank No. KF477317)), NJHA strain (GenBank No. KF477318), ZJDY strain (GenBank No. KF477319), NJPK strain (GenBank No. KF372027) and FJ17290 strain (GenBank No. MF576435). The genomes of these PTTVs were aligned using DNAStar software. The primers and probe were designed based on the conserved regions by using Primer Premier Software version 5.0 (Premier Biosoft, Palo Alto, CA, USA) and synthesized by TaKaRa (Dalian, China). The forward primer was PTTV-F (5′-CAAGGTATTCAACAAGTTAC-3′); the TaqMan probe was PTTV-P (FAM-5′-AACAGCTTGACCATTCCACGG-3′-Eclipse); the reverse primer was PTTV-R (5′-TGTGGGATTCCTGAA TAG-3′). The target amplicon was 81 base-pair in length. The designed primers and probe were evaluated with Blastn (BLAST; http://www.ncbi.nlm.nih.gov/blast/).

Real-time qPCR was preformed on Mastercycler ep realplex (Eppendorf, Germany) machine. Premix Ex Taq™ kit (Probe qPCR, TaKaRa, Dalian, China) with a final volume of 25 μl reaction was used in the study. The optimized reaction mixture consisted of 12.5 μl Premix Ex Taq, 1 μl PTTV-F (5 μmol/l), 1 μl PTTV-R (5 μmol/l), 2 μl PTTV-P (5 μmol/l), 2 μl template, and 6.5 μl Nuclease-Free Water (Ambion, Thermo Fisher Scientific, Beijing, China), respectively. The optimized

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https://doi.org/10.1016/j.mcp.2018.04.001
Received 28 February 2018; Received in revised form 26 March 2018; Accepted 2 April 2018

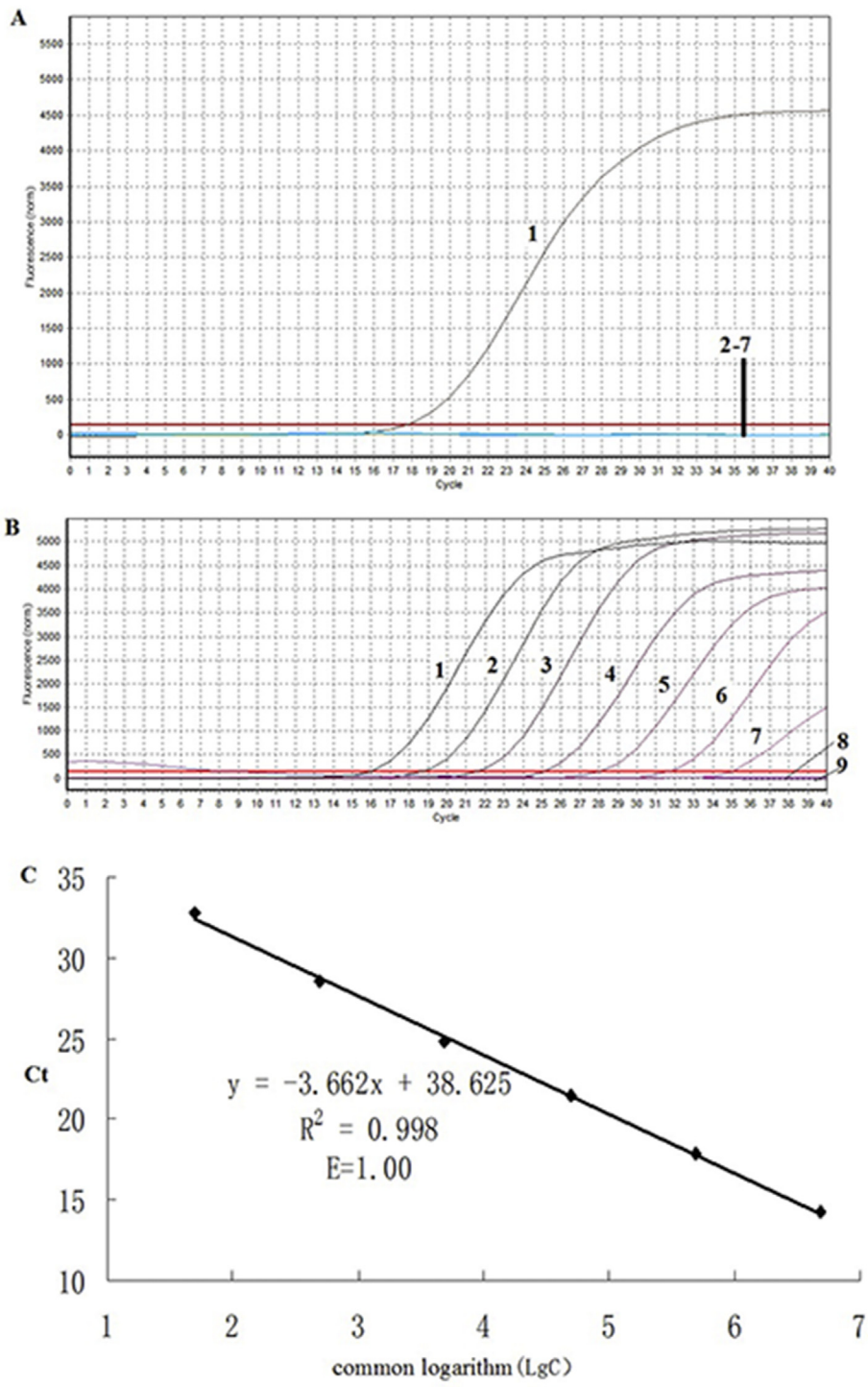

Fig. 1. A. The specificity test for qPCR assay of PTTV.
The results demonstrated only PTTV can be observed with positive fluorescence signal (lane 1), no positive fluorescence signal can be observed with other controls, including lane 1, PTTV; lane 2, PiCV; lane 3, DHV-1; lane 4, ATmV; lane 5, APMV-1; lane 6, CoHV-1; lane 7, Negative control (Nuclease-Free Water).
B. The sensitivity test for qPCR assay of PTTV.
The lowest copy number that could be determined by qPCR was up to 4.93×10^1 copies/μl (lane 7). Lane 1 to lane 7 means the templates with the concentration from 4.93×10^7 to 4.93×10^1 copies/μl; lane 8, 4.93×10^0 copies/μl; lane 8, Negative control (Nuclease-Free Water).
C. The standard curve for qPCR assay of PTTV.
Mean threshold cycle values (Ct) from three replicates (y-axis) are plotted versus common logarithmic (Lg C) concentrations of plasmid copies (x-axis).

qPCR procedure was initiated for 1 min at 95 °C, followed by 40 cycles of 10 s at 95 °C, 10 s at 58 °Cand 15 s at 72 °C. All reactions were conducted in triplicates.

To evaluate the qPCR specificity, various pigeon-origin viruses including pigeon circovirus (PiCV, fj1 strain) [8], pigeon-originated duck hepatitis A virus 1 (DHV-1, FJ1220 strain) [9]. Avian tembusu virus (ATmV, WR strain) [10] and Muscovy duck origin avian paramyxovirus type 1 (APMV-1, XBT14 strain) [11] which were used as controls instead of pigeon-origin tembusu virus and avian paramyxovirus type 1. The genomic DNA of columbid herpesvirus 1 (CoHV-1, BJ strain, GenBank: KU844281) [12] was kindly donated by Professor Jingliang Su. Viral DNA and RNA were extracted using the TIANamp Virus DNA kit (Tiangen, Beijing, China) and Trizol Reagent (Thermo Fisher Scientific, Beijing, China), respectively. The isolated RNAs were