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In vitro growth kinetics and gene expression analysis of the turkey adenovirus 3, a siadenovirus

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ABSTRACT

Turkey adenovirus 3 (TAdV-3) belongs to the genus *Siadenovirus*, family *Adenoviridae*. Previously, nucleotide sequencing and annotation of the Virginia avirulent strain (VAS) of TAdV-3 genome, isolated in our laboratory, indicated the presence of a total of 23 genes and open reading frames (ORFs). The goals of this study were 1) to delineate the growth kinetics of the virus using a qPCR-based infectivity assay, and 2) to determine the virus gene expression profile during the early and late phases of infection in target B lymphocytes. The one-step growth curve experiment demonstrated 3 phases of virus replication cycle: a lag phase lasted for 12–18 h post-infection (h.p.i.), in which the virus titer declined; a log phase from 18 to 120 h.p.i., in which the number of infectious virus particles increased over 20,000 folds, and a brief decline phase thereafter. Southern blot analysis indicated that the synthesis of new viral DNA started by 8 h.p.i. Gene-specific RT-PCR analysis revealed the expression of mRNAs from the 23 TAdV-3 genes/ORFs. According to the temporal transcriptional profiling of TAdV-3 genome, genes could be divided into 3 groups based on the time of transcription initiation: group 1 showed detectable levels of transcription at 2 h.p.i and included 7 genes, *i.e.*, hyd, III, pX, pVI, II, 100 K, and 33 K; group 2 included 12 genes whose mRNAs were detected for the first time at 4 h.p.i., *i.e.*, ORF1, IVa2, pol, pTP, pIIIa, EP, DBP, E3, U exon, IV, ORF7, and ORF8; group 3 of transcripts were detectable starting 8 h.p.i. and included only 4 genes, *i.e.*, 52 K, 22 K, pVII, and pVIII. Our data suggest that the transcriptional kinetics of genus *Siadenovirus* differ from that observed in other adenoviral genera; however, a few TAdV-3 genes showed similar expression patterns to their adenoviral homologs.

1. Introduction

Turkey adenovirus 3 (TAdV-3), more commonly known as turkey hemorrhagic enteritis virus (THEV), is the causative agent of a variety of clinical conditions in a number of avian species. TAdV-3 causes hemorrhagic enteritis in turkeys, marble spleen disease in pheasants, and avian adenovirus splenomegaly in chickens (Pierson and Fitzgerald, 2013). TAdV-3 targets IgM-bearing B lymphocytes, inducing apoptosis and transient immunosuppression, which often results in additional mortality due to secondary bacterial infection (Pierson and Fitzgerald, 2013; Pierson et al., 1996; Rautenschlein et al., 2000; Saunders et al., 1993; Suresh and Sharma, 1995, 1996). TAdV-3 has a non-enveloped, icosahedral capsid of 70–90 nm in diameter, enclosing a linear, non-segmented, double-stranded DNA (dsDNA) genome of ~26 kb (Beach et al., 2009; Iltis et al., 1977; Jucker et al., 1996; Tolin and Domermuth, 1975; van den Hurk, 1992). TAdV-3 belongs to the family *Adenoviridae*, genus *Siadenovirus*, species Turkey siadenovirus A (Adams et al., 2014). TAdV-3 and frog adenovirus 1 (FrAdV-1) have been the only two members in the genus until 2009. Since then, several siadenovirus members have been recognized and associated with infections in different avian and non-avian species (Katoh et al., 2009; Kovács and Benkő, 2009; Kovács et al., 2010; Rivera et al., 2009; Wellehan et al., 2009).

The first siadenoviral genome to be partially and fully sequenced was TAdV-3 (Beach et al., 2009; Jucker et al., 1996; Pitcovski et al., 1998), followed by FrAdV-1 (Davison et al., 2000) and other

Abbreviations: TAdV-3, turkey adenovirus 3; VAS, Virginia avirulent strain; ORFs, open reading frames; h.p.i., hour(s) post-infection; pol, adenovirus DNA-dependent DNA polymerase; IPV, infectious viral particle(s)

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