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Phylogenetic analysis, genomic diversity and classification of M class gene segments of turkey reoviruses

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ABSTRACT

From 2011 to 2014, 13 turkey arthritis reoviruses (TARVs) were isolated from cases of swollen hock joints in 2–18-week-old turkeys. In addition, two isolates from similar cases of turkey arthritis were received from another laboratory. Eight turkey enteric reoviruses (TERVs) isolated from fecal samples of turkeys were also used for comparison. The aims of this study were to characterize turkey reovirus (TRV) based on complete M class genome segments and to determine genetic diversity within TARVs in comparison to TERVs and chicken reoviruses (CRVs). Nucleotide (nt) cut off values of 84%, 83% and 85% for the M1, M2 and M3 gene segments were proposed and used for genotype classification, generating 5, 7, and 3 genotypes, respectively. Using these nt cut off values, we propose M class genotype constellations (GCs) for avian reoviruses. Of the seven GCs, GC1 and GC3 were shared between the TARVs and TERVs, indicating possible reassortment between turkey and chicken reoviruses. The TARVs and TERVs were divided into three GCs, and GC2 was unique to TARVs and TERVs. The proposed new GC approach should be useful in identifying reassortant viruses, which may ultimately be used in the design of a universal vaccine against both chicken and turkey reoviruses.

1. Introduction

Avian reoviruses (ARVs) are non-enveloped viruses with icosahedral symmetry and belong to genus *Orthoreovirus* in the family *Reoviridae* (Varela and Benavente, 1994). The ten double stranded RNA genomic segments are divided into three classes namely large (L), medium (M), and small (S), based on their migration patterns in polyacrylamide gel electrophoresis (Varela and Benavente, 1994; Benavente and Martinez-Costas, 2007). The L and M genes are further subdivided into three segments (L1, L2, L3 and M1, M2, M3, respectively) while the S gene is subdivided into four segments (S1, S2, S3, S4; Spandidos and Graham, 1976). The viral genome has 12 open reading frames (ORFs), which encodes for eight structural and four non-structural proteins. The structural proteins are an important part of the progeny virions while non-structural proteins are expressed only in infected cells (Martinez-Costas et al., 1997).

The L, M and S gene segments encode lambda (λ), mu (μ) and sigma (σ) proteins, respectively. The M1 and M2 segments encode two structural proteins (μA and μB, respectively) while M3 gene segment encodes a non-structural protein (μNS), which has an important role in the early stages of virus morphogenesis (Touris-Otero et al., 2004; Benavente and Martinez-Costas, 2007). The μA

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protein is a minor component of the inner capsid and is believed to serve as a putative transcriptase co-factor. The μB protein is present in the outer capsid and is important for virus penetration into the host cell after it is cleaved into μBN (small myristolylated amino-terminal peptide) and μBC (large carboxy-terminal fragment) (Benavente and Martinez-Costas, 2007).

The genus *Orthoreovirus* includes two types of reoviruses e.g., fusogenic and non-fusogenic. The fusogenic reoviruses induce cell-to-cell fusion resulting in the formation of syncytia in infected cells. Most mammalian reoviruses (MRVs) are non-fusogenic in nature except for the Nelson Bay reovirus (NBV; recently re-named as *Pteropine orthoreovirus* or PRV), bat, and baboon reoviruses. The orthoreoviruses are divided into five groups (I–V) (King et al., 2012); group I includes prototypical MRV strains; group II contains ARVs; group III includes PRV; and groups IV and V include baboon and reptilian orthoreoviruses, respectively.

The ARVs are ubiquitous in domestic poultry with 80% of them being non-pathogenic and are frequently found in clinically healthy birds (Jones, 2008). However, ARVs have also been known to cause different disease conditions in poultry including enteritis, hepatitis, neurological disorder, myocarditis, respiratory distress and viral arthritis/tenosynovitis (Jones, 2008). It is believed that ARV-associated clinical disease is mostly dependent on the age and immune status of the host and on the pathotype of the virus. Economic losses associated with ARVs in commercial poultry are due to poor weight gain, uneven growth, poor feed conversion, increased morbidity and mortality, and reduced marketability (Jones, 2008).

The ARVs of chickens (chicken reovirus or CRV) and their pathogenesis have been well defined (van der Heide and Kalbac, 1975; Al Afaleq and Jones, 1989). At least 11 serotypes and pathotypes of CRV exist (Wood et al., 1980; Jones, 2008), which have been implicated mainly in tenosynovitis and runting-stunting syndrome. Recently, CRV variants have been isolated from cases of lameness and tenosynovitis in 2.5–8 week old commercial broiler chickens in Europe and North America. Most of the commercially available vaccines are not effective against these variants (Rosenberger et al., 2013a; Sellers et al., 2013; Troxler et al., 2013).

The ARVs of turkeys (turkey reoviruses or TRVs) have been isolated not only from apparently healthy poults but also from cases of poult enteritis complex (PEC), poult enteritis syndrome (PES), and light turkey syndrome (LTS) (Pantin-Jackwood et al., 2008; Mor et al., 2013a; Jindal et al., 2010, 2014). In 1980s, TRVs were isolated from arthritic joints of turkeys (Levisohn et al., 1980; Page et al., 1982; Al Afaleq and Jones, 1990). After this, subsequent reports of arthritis-associated reoviruses in turkeys were lacking. However, we recently reported on the isolation of reoviruses from tendons of 12–18-week-old market age tom turkeys from the upper Midwest area of the U.S. On experimental inoculation in turkey poults, these viruses caused tenosynovitis and were named turkey arthritis reoviruses (TARVs) to differentiate them from turkey enteric reoviruses (TERVs) isolated from healthy and enteritic poults (Mor et al., 2013b, 2014b; Sharafeldin et al., 2014).

The reoviruses isolated from ducks and geese are called duck reovirus (DRV) and goose reovirus (GRV), respectively. In addition, ARVs have been isolated from wild birds. For example, Tvarminne avian virus (TVAV) was isolated from the brain of a wild crow with neurological signs (Dandár et al., 2014b). We isolated an ARV from a chickadee affected with enteritis. Sequencing of S4 gene segment of this virus (Chickadee/2011/USA/MN/KJ475124) revealed a close relationship with TRVs (Mor et al., 2014a).

While the M class gene segments of CRV, DRV, and GRV have been described, this information is not available for either TERV or TARV while the S class genome segments has been characterized for TARVs (Noad et al., 2006; Su et al., 2006; Zhang et al., 2007; Bányai et al., 2011; Wang et al., 2013; Dandár et al., 2014a; Mor et al., 2014b). Based on complete S class analysis, divergence was found within TRVs and among TRVs, CRVs, DRVs and GRVs. The criteria established by the International Committee on Taxonomy of Viruses (ICTV) for species demarcation include nucleotide identity between homologous genome segments of >75% within species versus <60% between species for most of the segments. Lui et al. (2003) proposed 10% nucleotide divergence criteria to define lineages of CRVs variants, which is currently being used for defining lineages of CRVs, DRVs and GRVs (Su et al., 2006; Zhang et al., 2007; Wang et al., 2013). We propose here a new genotype classification of ARVs based on nucleotide cut-off value for each M class gene segment to further characterize them.

2. Materials and methods

2.1. Sample source

Dead or humanely euthanized lame turkeys (2–19 weeks of age) or fresh or frozen turkey legs from lame turkeys were submitted to the University of Minnesota Veterinary Diagnostic Laboratory (UMVDL), St. Paul, MN. From tendons and/or joint fluids of these cases, we isolated TARVs in QT-35 cells as previously described (Mor et al., 2013b). For histopathology, gastrocnemius and digital flexor tendons were removed and immersed in 10% buffered formalin and processed as previously described (Sharafeldin et al., 2014). Fecal samples from enteritic turkeys were also processed for isolation of TERVs as previously described (Jindal et al., 2010; Mor et al., 2013a).

2.2. Virus isolates

A total of 15 TARV isolates were included in this study. Thirteen TARV strains (TARV-MN1 to TARV-MN13) were isolated at the UMVDL and two trains (TARV-O'Neil and TARV-Crestview) were obtained from Dr. Jack Rosenberger, AviServe, Newark, Delaware. In addition, eight TERV strains (TERV-MN1 to TERV-MN8) were isolated from fecal samples and used for comparison. All viruses were isolated and propagated in QT-35 cells as described previously (Mor et al., 2013b, 2014b).