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Short communication

Detection of a mammalian-like astrovirus in bird, European roller (*Coracias garrulus*)

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ABSTRACT

Astroviruses are small, non-enveloped viruses with positive sense, single-stranded RNA genomes. The family *Astroviridae* contains two genera, *Mamastrovirus* and *Avastrovirus*, which – based upon our current knowledge – infect mammals and birds, respectively. However, recent seroprevalence study indicated that people with contact to turkeys can develop serological responses to the turkey astrovirus and minks might have been infected with the avastrovirus. These data suggest that the "host species/astrovirus genus" association should be permeable; however, mamastrovirus infection has not been reported from avian species, yet. In this study, a novel astrovirus was identified by viral metagenomics and RT-PCR methods in 2 (11%) out of 19 faecal samples collected from a wild, carnivorous bird species, European rollers (*Coracias garrulus*) from two breeding territories in Hungary. The complete genome sequence of astrovirus Er/SZAL6/HUN/2011 (KP663426) was 7025 nt-long and had some unique genomic features including an unusually long spacer between the subgenomic RNA promoter and the ORF2 initiation codon. Using the BLASTp Er/SZAL6/HUN/2011 had the highest aa identities 35%, 61% and 34% to *MAstV 32* (JF713710, host: porcine), to *MAstV 23* (JF729316, host: rabbit) and to unclassified porcine astrovirus (JX684071) in ORF1a, ORF1b and ORF2, respectively. The same proteins of Er/SZAL6/HUN/2011 had 25%, 66% and 33% aa identities to the corresponding proteins of murine astrovirus (JX544743) as the closest strain. The sequence- and phylogenetic analysis indicated that Er/SZAL6/HUN/2011 represents the first member of a novel mamastrovirus species. Data suggest that both mammals and birds could have been exposed to mamastroviruses and avastroviruses providing opportunities for cross-species infection and viral adaptation with cross-class astroviruses especially in carnivorous animals. Further investigation is needed to determine the origin, natural host species spectrum, distribution and spread of Er/SZAL6/HUN/2011 among vertebrates.

1. Introduction

Astroviruses, first identified in 1975 (Appleton and Higgins, 1975), are the third common cause of gastroenteritis in humans. At present, the family *Astroviridae* consists of two genera *Mamastrovirus* (*MAstV*) and *Avastrovirus* (*AAstV*) with 19 and 3 officially ratified species (Guix et al., 2013; Bosch et al., 2014; http://www.iah-virus.org/astroviridae/). The number of newly discovered astroviruses is rapidly increasing and many are still waiting for classification (Guix et al., 2013; http://www.iah-virus.org/astroviridae/).

Astroviruses are small (28–30 nm), non-enveloped viruses with positive sense, single-stranded RNA (+ssRNA) genomes with an average of 6.400–7.900 nucleotides (nt) (De Benedicts et al., 2011; Knowles et al., 2012; Méndez et al., 2013; Bosch et al., 2014). The genome is arranged in three open reading frames (ORFs), although an alternative ORF X was described in human and some mammalian astroviruses (Firth and Atkins, 2010). The ORF1a and ORF1b encode the non-structural proteins while ORF2 encodes the viral capsid structural proteins. The general organization of the genome begins with a relatively small (11–85 nt) 5′untranslated region (UTR) followed by the ORF1a coding region consisting of several coiled coil secondary RNA structural elements: transmembrane domains (TM) and potential nuclear

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localization signals (NLS). The ORF1a also encodes a serine protease enzyme (Knowles et al., 2012; Méndez et al., 2013) and a viral protein genome-linked (VPg) (Fuentes et al., 2012). The putative VPg protein is depicted as bound to both the 5′end of the genomic- and subgenomic RNA (sgRNAs) (Fuentes et al., 2012; Méndez et al., 2013), respectively. ORF1b encodes an RNA-dependent RNA polymerase (RdRp) with conserved amino acid motifs (Koonin, 1991). Between the ORF1a and ORF1b there is a conserved heptameric ribosomal frameshift signal (AAAAAAC) forming a downstream stem-loop (SL) structure (Jiang et al., 1993; Méndez et al., 2013). The third coding region (ORF2) is overlapped with ORF1b and encodes the viral capsid protein. During the RNA replication ORF2 is expressed from a sgRNA (Méndez et al., 2013). The sgRNA promoter sequence has a highly conserved nucleotide sequence motif and is present upstream the ORF2 start codon (Méndez et al., 2013). The nucleotide (nt) sequence region between the sgRNA promoter sequence and the ORF2 initiation start codon is varies in length, but thought to be less than 11 nt (Reuter et al., 2012a; Méndez et al., 2013). The astrovirus genome ends with 3′UTR followed by a poly(A)-tail (Knowles et al., 2012; Méndez et al., 2013). In some astroviruses the ORF2/3′UTR junction region contains stem-loop secondary RNA structures and conserved nucleotide stem-loop-2-motif (s2m) (Knowles et al., 2012; Guix et al., 2013; Pantin-Jackwood et al., 2013; Méndez et al., 2013).

At the time, mamastroviruses were identified in mammals such as human, ovine, bovine, porcine, wild boar, rabbit, canine, feline, murine, mink, insectivorous bat, cheetah, California sea lion, steller sea lion, bottlenose dolphin, brown rat, roe deer and red fox species (Knowles et al., 2012; Cattoli et al., 2013; Guix et al., 2013; Bosch et al., 2014; http://www.iah-virus.org/astroviridae/). On the other hand, avastrovirus was found in some avian species like chicken, duck, turkey, guinea fowl, pigeon and aquatic birds (De Benedicts et al., 2011; Knowles et al., 2012; Pantin-Jackwood et al., 2013; Guix et al., 2013; Chu et al., 2012; http://www.iah-virus.org/astroviridae/). Serological evidence against avastrovirus has been found recently in humans (Meliopoulos et al., 2014) and avastrovirus has been identified in minks (Sun et al., 2014) suggesting a potential cross-species transmissions of avastrovirus in birds and mammals. However, *Mamastrovirus* has not been detected in avian species. The astrovirus host species is highly diverse; therefore the characterization of astrovirus diversity in different host species could be helpful to understand their evolution, origin and cross-species transmission.

Our knowledge about viruses in birds especially in wild birds is still limited or underestimated compared to the thousands of known bird species (>10,000). The free-living birds could be a natural host and disperser of different microbes, including viruses. The long-distance migrant wild bird European roller (*Coracias garrulus*) winters in sub-Saharan Africa, but breeds in Europe, is a predator bird that feeds on almost smaller than its size, large insects, lizards, small snakes, rodents, shrews (Fry et al., 2014; http://www.birdlife.org).

This study describes the first identification and complete genome characterization of a novel mamastrovirus from faecal samples collected from European rollers in Hungary.

2. Materials and methods

Faecal samples ($N = 19$) were collected from clinically healthy European rollers (*C. garrulus*) in two breeding territories (Dorozsma-Majsai homokhát, $N = 15$ and Borsodi Mezőség, $N = 4$) of the Great Hungarian Plain, Hungary, in July 2011. Specimens were collected from natural nests during regular bird ringing by qualified ornithologists with valid permission (Permit No. NIFENW14/1368-5/2011). One randomly selected faecal sample from each breeding territory was chosen to viral metagenomic analysis (454 GS FLX technology) as reviewed previously (Kapoor et al., 2008; Victoria et al., 2009; Boros et al., 2013; Reuter et al., 2014; Pankovics et al., 2015). The assembled sequence contigs were compared to the GenBank nucleotide and protein database using BLASTn/BLASTp. Specific primers were designed to the contigs in order to obtain the complete viral genome by primer-walking, 5′/3′ RACE, dsRNA-RACE and TAIL-PCR methods (Liu and Chen, 2007; Boros et al., 2012; Pankovics et al., 2015). PCR-products were sequenced directly using the specific primers then run on an automated sequencer (ABI PRISM 310, Applied Biosystems, Stafford, USA).

Astroviruses were screened by RT-PCR using a common screening (Chu et al., 2008; Kapoor et al., 2009) and strain specific (SZAL6A-scr-R: 5′-GGCTTTACCCACATACCAAA-3′, SZAL6A-scr-F: 5′-GAGTTTGACTGGACCCGTTTTGA-3′) primer pairs. The pairwise distance (p-distance) amino acid (aa) analysis between representative astroviruses and the study strain were estimated by bootstrap method ($N = 1000$ replicates) and partial deletion (95%) with MEGA6 (Tamura et al., 2013). Representative and complete ORF1a, ORF1b and ORF2 astrovirus aa sequences were pre-tested with the best aa model (ML) search and dendrograms were constructed by the maximum-likelihood method based on the Le_Gascuel_2008 model using MEGA6 (Tamura et al., 2013). The ORFs were determined by the NCBI ORF finder. The secondary RNA structures were predicted by MFold (Zuker, 2003), the prediction of transmembrane domains and nuclear localization signals were analyzed by TMHMM program (https://www.cbs.dtu.dk/services/TMHMM/) and cNLS Mapper (Kosugi et al., 2009; http://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi), respectively. The protein folding prediction was carried out using FoldIndex (http://bip.weizmann.ac.il/fldbin/findex). The novel mamastrovirus was named after the host species European roller to ErAstV. The novel astrovirus sequences Er/SZAL6/HUN/2011 and Er/BMTK529/HUN/2011 were submitted to GenBank under accession number KP663426 and KR057184.

3. Results

In one (“SZAL6”) of the two faecal samples five assembled astrovirus sequence contigs, two from ORF1a, one from ORF1b and two from ORF2, were found using viral metagenomics (Fig. 1A). The complete genome of Er/SZAL6/HUN/2011 (KP663426) is 7025 nt long, excluding the poly(A) tail and has a common astrovirus genome RNA organization (Fig. 1). The ORF1a, ORF1b and the ORF2 regions of Er/SZAL6/HUN/2011 are 2823 nt, 1482 nt and 2769 nt long (Fig. 1A) and encode three potential protein precursors of 941 aa, 494 aa, 923 aa, respectively. The hypothetical ORF X was not found (Firth and Atkins, 2010). Er/SZAL6/HUN/2011 shares 52.7% G + C content and it has 26.5% A, 20.8% U, 25% G and 27.7% C nt distribution.

The 5′UTR is 19 nt long and no conserved promoter sequence was observed. The putative in-frame AUG initiation codon of ORF1a is at nt position 20–22 (ACG**A_{20}UG**G) showing the ANNAUGG consensus Kozak context (Kozak, 1987). The ORF1a showed the closest match to trypsin-like peptidase domain by conserved domain search (CDS) (Fig. 1B). Two potential transmembrane domains (aa 297–319 and aa 358–380) were predicted in the N-terminal half of Er/SZAL6/HUN/2011 ORF1a. Two possible nuclear localization signals were found; one of the two (R_{500}TFPGRDIALLKMPHPLQGAIQRLKIAKTPKYD) is located between the last TM domain and the predicted serine protease and the other (R_{675}TAGRKKNKRGRGRMKTQPGYHHAARRRQRGPM) is part of the predicted VPg aa sequence (Fig. 1B). The ORF1a also encodes a

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