Detection and genetic characterization of a novel pig astrovirus: relationship to other astroviruses

Marc-André Laurin · Margaux Dastor · Yvan L’Homme

Received: 13 June 2011 / Accepted: 30 July 2011 / Published online: 8 September 2011 © Her Majesty the Queen in the Right of Canada as represented by the Minister of the Environment 2011

Abstract Emerging viruses represent a continuous threat to human health and to farmed animals, as evidenced on multiple occasions by outbreaks of influenza, henipavirus and SARS. Knowledge about the diversity of viromes present in reservoir species can lead to a better understanding of the origin of emerging pathogens. In this study, we extend the knowledge of astrovirus diversity in pigs by reporting the genetic characterization of an unknown astrovirus lineage. Phylogenetic analyses provided evidence that this porcine astrovirus lineage is unique and does not appear to share a recent common ancestor with any known mamastrovirus. The data reported in this study extend the number of porcine astrovirus lineages to a total of five, all of which most likely represent distinct species of different origins.

The family Astroviridae consists of small (28–30 nm), non-enveloped, single-stranded positive-sense RNA viruses of approximately 7 kb in length. These viruses generally exhibit a distinctive five- or six-pointed star-shape appearance when viewed using electron microscopy. The AstV genome is arranged into three open reading frames (ORFs) designated ORF1a, ORF1b and ORF2. ORF1a and ORF1b are situated at the 5’ end of the genome and encode non-structural polyproteins, including a protease and an RNA-dependent RNA polymerase (RdRp). ORF2, situated at the 3’ end of the genome, encodes the structural capsid protein and is transcribed as a subgenomic mRNA [18, 19].

The family Astroviridae is separated into two genera; viruses of the genus Mammastrovirus infect mammals, and viruses of the genus Avastrovirus are found in avian hosts [19]. Mammastroviruses appear to have a broad host range, since they have been isolated from numerous host species, including humans, mink, sheep, pigs, rats, marine mammals, dogs, cheetahs, roe deer, cattle and bats [2, 4, 14, 19, 21, 24–26, 28]. The list of susceptible species is likely to continue growing as more species are investigated.

Astroviruses are generally associated with either mild or severe enteric disease symptoms such as diarrhea and vomiting in a number of mammalian species [18]. Human astrovirus (HuAstV) serotypes 1–8, which are the most extensively studied astroviruses, are known to be a common cause of diarrhea in young children, the elderly and the immunocompromised [1, 6, 11, 12]. In addition, a number of genetically distinct strains of HuAstV have recently been identified, characterized and proposed to represent novel AstV species based on genetic distance criteria [8, 15].

Porcine AstVs (PoAstV) were first detected by EM in the feces of a diarrheic piglet [3] and later isolated in culture [23]. Molecular characterization of the ORF2 gene from this isolate followed some years later [14]. In the last five years or so, different research groups have successfully used PCR approaches to investigate the presence and diversity of porcine astroviruses [13, 17, 20]. Collectively, these studies have unveiled an impressive genetic diversity among PoAstV strains. This diversity suggests different
origins for the various PoAstV lineages and, presumably, a number of transspecies transmission events between susceptible mammalian hosts [14, 17]. In the present study, we report the identification and characterization of unforeseen and divergent PoAstV strains. Phylogenetic analysis of the 3’ end of this strain suggests that a novel and possibly fifth lineage of AstV exists in swine and heightens concerns about the role of pigs as a potential source of emerging astrovirus infections.

A total of 48 fecal samples originating from the individual cecal content of slaughtered adult pigs in Canada were collected in 2008–2009. Viral RNA was extracted as described previously and stored at −70°C until needed [17]. The general strategy employed for obtaining PoAstV sequences is summarized in Fig. 1. Briefly, primers panAV-F11 (forward), panAV-F12 (forward) and panAV-R1 (reverse) were originally designed by Chu and colleagues based on a conserved region situated in the RNA-dependent RNA polymerase (RdRp) gene of AstVs [5]. PCR conditions using these primers were as described by Luo and colleagues [17]. PCR products were analyzed on a Qiaxcel instrument (QIAGEN, Mississauga, Ontario, Canada) and sequenced in both directions using the big dye v3.1 chemistry on a 3730xl instrument from Applied Biosystems (Foster City, CA). Gel-purified amplicons were cloned using a TOPO 2.1 (T/A) cloning kit (Invitrogen, Mississauga, Ontario, Canada) and sequenced in both directions using a primer walking strategy. Sequence editing, assembly and analysis were performed using BioEdit version 7.0.9.0 (http://www.ncbi.nlm.nih.gov/blast). Multiple sequence alignments were constructed with CLUSTAL W (version 1.6). Phylogenetic analysis was conducted using the neighbor-joining (NJ) method with p-distances for nucleotides and a Poisson distance correction calculation for amino acids using the Molecular Evolutionary Genetic analysis (MEGA 4.0) software with default settings, except that all missing data or gaps were completely ignored. Confidence values at the nodes were obtained by performing 1,000 bootstrap analyses.

We have previously reported the detection and characterization in Canadian pigs of novel and previously unknown AstVs belongs to the genus Mamastrovirus using a “broad-range” PCR strategy [17]. We hence applied the same strategy to screen a collection of 48 fecal samples from pigs slaughtered in 2008–2009. A total of 39 samples generated amplicons of the expected size, which were cloned and sequenced. Putative amino acid sequences from all strains revealed the presence of the characteristic “YGDD” motif located near the C-terminal region of the RdRp (not shown). This motif has been reported for all astrovirus polymerases characterized thus far. BLAST analysis revealed that most sequences were, in fact, closely related (>85% nucleotide identity) to known porcine AstV sequences previously characterized by Luo and colleagues [17]. However, a group of four related sequences revealed only approximately 70% nucleotide identity to bat and human AstV sequences in the database, suggesting that these sequences were divergent from known PoAstV strains and possibly novel. Pairwise identity comparisons of the partial RdRp nucleotide sequences revealed that the four strains were 80–90% identical to each other. Phylogenetic analysis of these sequences, in addition to prototypical animal AstV strains, confirmed their relatedness and grouped them in a unique lineage on a divergent branch distantly related to other known mamastroviruses (PoAstV 5 in Fig. 2a).

To permit more in-depth genomic investigation and strengthen their taxonomic grouping, we performed 3’ RACE-PCR on these four PoAstV 5 samples. We were able to amplify and characterize a 3029 nt-long sequence from strain PoAstV CC12 only. It is unclear why 3’ RACE-PCR failed with the other samples. The amplified region from strain PoAstV CC12 included the 3’ end of the RdRp gene, the complete capsid gene, and the 3’ UTR (Fig. 1). The conserved motif situated at the junction between ORF1b and ORF2, which is thought to represent a regulatory element serving as a promoter for subgenomic RNA transcription, was present in strain PoAstV CC12: TTGGGGGGGAGGACCCAAAAAGAGACGAGCCG (following the convention of HuAstV, which places the initiation ATG codon, underlined, for ORF2 immediately upstream of the ORF1b stop codon) [18]. The ORF2 start codon of strain CC12, underlined, appeared in an optimal Kozak context for translation initiation (RNNAUUGG, where R = A/G and N = A/T/G/C) [16]. The ORF2 gene is predicted to encode a capsid protein 735 amino acids...
long, which is comparable to most AstVs. The length of the 3′UTR is 101 nucleotides. The highly conserved stem-loop-II-like motif (s2 m) present in the 3′UTRs of most mammastroviruses is also present in strain PoAstV CC12 (not shown). This motif has been suggested to have an important function in viral RNA replication [14].

Phylogenetic analysis of the complete capsid coding region of PoAstV CC12 confirms that this strain forms a distinct lineage in the family Mamastroviridae, including previously identified porcine astroviruses from different continents (Fig. 2b). In addition, the tree topology revealed by our analysis is largely in agreement with previous studies [7–10, 15, 17, 21]. Pairwise amino acid comparisons reveal that strain PoAstV CC12 shares only between 25% and 30% aa identity in the complete ORF2 with prototypical AstV strains. Since phylogenetic analysis...
placed the CC12 strain in a unique lineage, we named this strain PoAstV 5, as there now appear to be five distinct lineages of AstVs in swine [13, 14, 17, 20], and this name maintains the continuity in the nomenclature of PoAstV strains. The genetic distance between PoAstV CC12 and the other strains is comparable to distances between members of established AstV species, suggesting that CC12 possibly represents a new, fifth PoAstV species.

Until recently, a relatively small number of astroviruses from very few hosts were known [18]. However, in the last few years, thanks in part to metagenomic analyses and broad-range PCR, a number of studies have revealed a wide array of divergent AstV strains in a growing number of mammalian species [4, 14, 15, 21, 24, 26–28]. The present work extends current knowledge about these agents and underscores the vast diversity and divergence of astrovirus strains harbored by swine [13, 17, 20]. PoAstVs now appear in five distinct lineages of the AstV evolutionary tree, which suggests different ancestral origins and numerous interspecies transmissions involving many mammalian species, including humans. As additional mammalian species are screened for the presence of AstVs, a clearer picture of the historical transmission path used by these viruses between different hosts might emerge and shed new light on the zoonotic potential of these viruses.

Emerging pathogens represent a constant threat to human health. Since the majority of these pathogens arise from animal reservoirs, a more thorough knowledge about the presence and diversity of viruses in animal species such as pigs could lead to a better characterization of the risk posed by such agents. In addition, a better understanding and appreciation of the virome present in wild and domestic animals could lead to early identification of the source of an emerging outbreak and therefore to faster and more targeted interventions to control and limit the spread of a disease. The discovery of novel PoAstV strains described here provides an example of how diverse these viruses are in this domestic reservoir species.

Acknowledgment This work was supported by the Science Division of the Canadian Food Inspection Agency (CFIA).

References

1. Akihara S, Phan TG, Nguyen TA, Hansman G, Okitsu S, Ushijima H (2005) Existence of multiple outbreaks of viral gastroenteritis among infants in a day care center in Japan. Arch Virol 150:2061–2075
2. Atkins A, Wellehan JF Jr, Childress AL, Archer LL, Fraser WA, Citino SB (2009) Characterization of an outbreak of astroviral diarrhea in a group of cheetahs (Acinonyx jubatus). Vet Microbiol 136:160–165
3. Bridger JC (1980) Detection by electron microscopy of caliciviruses, astroviruses and rotavirus-like particles in the faeces of piglets with diarrhoea. Vet Rec 107:532–533
4. Chu DK, Chin AW, Smith GI, Chan KH, Guan Y, Peiris JS, Poon LL (2010) Detection of novel astroviruses in urban brown rats and previously known astroviruses in humans. J Gen Virol 91:2457–2462
5. Chu DK, Poon LL, Guan Y, Peiris JS (2008) Novel astroviruses in insectivorous bats. J Virol 82:9107–9114
6. Dennehy PH, Nelson SM, Spangenberg S, Noel JS, Monroe SS, Glass RI (2001) A prospective case–control study of the role of astrovirus in acute diarrhea among hospitalized young children. J Infect Dis 184:10–15
7. Finkbeiner SR, Holtz LR, Jiang Y, Rajendran P, Franz CJ, Zhao G, Kang G, Wang D (2009) Human stool contains a previously unrecognized diversity of novel astroviruses. Virol J 6:161
8. Finkbeiner SR, Kirkwood CD, Wang D (2008) Complete genome sequence of a highly divergent astrovirus isolated from a child with acute diarrhea. Virol J 5:117
9. Finkbeiner SR, Le BM, Holtz LR, Storch GA, Wang D (2009) Detection of newly described astrovirus MLB1 in stool samples from children. Emerg Infect Dis 15:441–444
10. Finkbeiner SR, Li Y, Ruone S, Conardy C, Gregoricus N, Toney D, Virgin HW, Anderson LJ, Vinje J, Wang D, Tong S (2009) Identification of a novel astrovirus (astrovirus VA1) associated with an outbreak of acute gastroenteritis. J Virol 83:10836–10839
11. Gallimore CI, Taylor C, Gennery AR, Cant AJ, Galloway A, Iturriza–Gomara M, Gray JJ (2006) Environmental monitoring for gastroenteric viruses in a pediatric primary immunodeficiency unit. J Clin Microbiol 44:395–399
12. Gray JJ, Wreghitt TG, Cubitt WD, Elliot PR (1987) An outbreak of gastroenteritis in a home for the elderly associated with astrovirus type 1 and human calicivirus. J Med Virol 23:377–381
13. Indik S, Valicek L, Smid B, Dvorakova H, Rodak L (2006) Isolation and partial characterization of a novel porcine astrovirus. Vet Microbiol 117:276–283
14. Jonassen CM, Jonassen TO, Saif YM, Snodgrass DR, Ushijima H, Shimizu M, Grinde B (2001) Comparison of capsid sequences from human and animal astroviruses. J Gen Virol 82:1061–1067
15. Kapoor A, Li L, Victoria J, Oderinde B, Mason C, Pandey P, Zaidi SZ, Delwart E (2009) Multiple novel astrovirus species in human stool. J Gen Virol 90:2965–2972
16. Kozak M (1991) Structural features in eukaryotic mRNAs that modulate the initiation of translation. J Biol Chem 266:19867–19870
17. Luo Z, Roi S, Dastor M, Gallice E, Laurin MA, L’homme Y (2011) Multiple novel and prevalent astroviruses in pigs. Vet Microbiol 149:316–323
18. Mendez E, Arias CF (2007) Astroviruses. In: Knipe DM, Howley PM (eds) Fields virology. Lippincott Williams & Wilkins, Philadelphia, pp 981–1000
19. Monroe SS (2005) Astroviridae. In: Carter MJ, Herrmann J, Mitchell JK Sanchez-Fauquier A (eds) Virus taxonomy. Eighth report of the International Committee on Taxonomy of Viruses.Elsevier, Amsterdam, pp 859–864
20. Reuter G, Pankovics P, Boros A (2011) Identification of a novel astrovirus in a domestic pig in Hungary. Arch Virol 156:125–128
21. Rivera R, Nollens HH, Venn-Watson S, Gulland FM, Wellehan JF Jr (2010) Characterization of phylogenetically diverse astroviruses of marine mammals. J Gen Virol 91:166–173
22. Scotto–Lavino E, Du G, Frohman MA (2006) 3’ end cDNA amplification using classic RACE. Nat Protoc 1:2742–2745
23. Shimizu M, Shirai J, Narita M, Yamane T (1990) Cytopathic astrovirus isolated from porcine acute gastroenteritis in an established cell line derived from porcine embryonic kidney. J Clin Microbiol 28:201–206
24. Smits SL, Van LM, Kuiken T, Hammer AS, Simon JH, Osterhaus AD (2010) Identification and characterization of deer astroviruses. J Gen Virol 91:2719–2722
25. Toffan A, Jonassen CM, De BC, Schiavon E, Kofstad T, Capua I, Cattoli G (2009) Genetic characterization of a new astrovirus detected in dogs suffering from diarrhoea. Vet Microbiol 139:147–152

26. Tse H, Chan WM, Tsoi HW, Fan RY, Lau CC, Lau SK, Woo PC, Yuen KY (2011) Re-discovery and genomic characterization of bovine astroviruses. J Gen Virol 92(Pt 8):1888–1898

27. Zhu AL, Zhao W, Yin H, Shan TL, Zhu CX, Yang X, Hua XG, Cui L (2011) Isolation and characterization of canine astrovirus in China. Arch Virol 156(9):1671–1675

28. Zhu HC, Chu DK, Liu W, Dong BQ, Zhang SY, Zhang JX, Li LF, Vijaykrishna D, Smith GJ, Chen HL, Poon LL, Peiris JS, Guan Y (2009) Detection of diverse astroviruses from bats in China. J Gen Virol 90:883–887