RESEARCH REPORT
SWINE HEALTH

Title: Characterization of porcine astrovirus infection in the U.S. pig population – **NPB #12-189**

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Industry Summary

Many astrovirus (AstV) species are associated with enteric disease. In this study, the prevalence rates of porcine AstV types 1–5 (PAstV1–PAstV5) in the U.S. pig population were investigated using fecal samples from 509 pigs. Specifically, two multiplex differential PCR assays capable of detection and differentiation of all five known PAstV were developed. Among the 509 pigs tested, 488 (95.9%) came from farms with a history of diarrhea. All of the five known PAstV types were found to circulate in pigs in the U.S., and co-infection of a single pig with two or more PAstV types was frequently observed. A high overall prevalence of 64.0% (326/509) of PAstV RNA-positive samples was detected, with 97.2% (317/326) of the PAstV RNA-positive pigs infected with PAstV4. The first complete genome of a PAstV3 isolate was obtained and showed identities of 50.5–55.3% with mink AstV and the novel human AstVs compared with 38.4–42.7% with other PAstV types. Further extensive attempts to grow PAstV *in vitro* were unsuccessful. *In vivo* inoculation of pigs with PAstV RNA positive material resulted in short duration of PAstV shedding of low magnitude (less than a week) followed by seroconversion. Lesions were not detected in any of the pigs.

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Keywords: Porcine astrovirus; detection; isolation; pig model.

Scientific Abstract

Many astrovirus (AstV) species are associated with enteric disease. In this study, the prevalence rates of porcine AstV types 1–5 (PAstV1–PAstV5) in the U.S. pig population were investigated using fecal samples from 509 pigs. Specifically, two multiplex differential PCR assays were developed capable of detection and differentiation of all five known PAstV. Among the 509 pigs tested, 488 (95.9%) came from farms with a history of diarrhea. All of the five known PAstV types were found to circulate in pigs in the U.S., and co-infection of a single pig with two or more PAstV types was frequently observed. A high overall prevalence of 64.0% (326/509) of PAstV RNA-positive samples was detected, with 97.2% (317/326) of the PAstV RNA-positive pigs infected with PAstV4. The first complete genome of a PAstV3 isolate was obtained and showed identities of 50.5–55.3% with mink AstV and the novel human AstVs compared with 38.4–42.7% with other PAstV types. Further *in* vitro growth attempts of PAstV were unsuccessful. In *vivo* inoculation of pigs with PAstV RNA positive material resulted in short PAstV shedding of low magnitude (less than a week) followed by seroconversion. Lesions were not detected in any of the pigs.

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Introduction

Astroviruses (AstV) are small (28–30 nm), non-enveloped, single-stranded positive-sense RNA viruses of 6.4-7.7 kb in length, first identified in 1975 by electron microscopy in children suffering from diarrhea (Appleton and Higgins, 1975; de Benedictis et al., 2011; Fu et al., 2009). The AstV genome includes three open reading frames (ORFs) designated ORF1a, ORF1b and ORF2. ORF1a and ORF1b, situated at the 5' end of the genome, encode non-structural polyproteins including a protease and a RNA dependent RNA polymerase. ORF2 is situated at the 3' end of the genome, encodes the structural capsid protein, and is transcribed as a subgenomic mRNA (Mendez and Arias, 2007). The family *Astroviridae* has been classified into two genera, namely *Mamastroviruses* and *Avastroviruses* known to infect mammalian and avian species, respectively (Mendez and Arias, 2007).

Generally, enteric infections in humans caused by AstV have been reported worldwide mainly in infants and young children, and many studies suggest that astroviruses are the second most common cause of gastroenteritis in children after rotavirus infection (de Benedictis et al., 2011). Astrovirus infections in animals are also associated with mild-to-severe enteric disease characterized by diarrhea, vomiting, abdominal pain, and sometimes fever and immunosuppression (Moser and Schultz-Cherry, 2005). In some cases, AstV have been demonstrated in extra-enteric locations, such as with avian nephritis virus which is known to cause interstitial nephritis and growth retardation of young chickens (Imada et al., 2000) and the duck AstV that causes severe hepatitis and mortality rates up to 50% in ducklings (Fu et al., 2009; Gough et al., 1984). Mink AstV, associated with pre-weaning diarrhea, has recently also been associated with the central nervous system and the shaking mink syndrome (Blomstrom et al., 2010).

Porcine AstV (PAstV) was first detected by electron microscopy in association with post-weaning diarrhea in 3-week-old pigs in the UK (Bridger, 1980) and was later isolated by using an embryonic swine kidney established cell line and porcine kidney-15 (PK-15) cells. Limited experimental work with these early strains of PAstV indicates that they are able to induce mild diarrhea in piglets (Indik et al., 2006; Shimizu et al., 1990). Specifically, two cesarean-derived, colostrum-deprived (CDCD) pigs were infected with a PCR positive fecal sample obtained from pigs in the Czech Republic. The two experimentally infected pigs were euthanized after 6-7 days when they developed mild diarrhea, and astrovirus like virions were detected in the feces (Indik et al., 2006). In another small study conducted in Japan, three CDCD pigs infected with a cell culture suspension containing PAstV developed mild diarrhea (Shimizu et al., 1990).

The molecular characterization of PAstV has been limited to the 3' partial ORF1b and ORF2 genes for several years (Indik et al., 2006; Jonassen et al., 2001; Lan et al., 2011; Laurin et al., 2011; Luo et al., 2011; Reuter et al., 2011; Wang et al., 2001). The first whole genome of a Chinese PAstV 1 was published in September 2011 (GQ914773).

Objectives

The objectives of this study were:

I. To further validate a multiplex real-time PCR for PoAstV with the ability to further differentiate between subtypes which will be used to investigate the prevalence of PoAstV in postweaning diarrhea cases in the U.S.

II. To propagate PoAstV in cell culture or via pig passage and experimentally infect colostrum-deprived pigs via stomach tube to investigate the pathogenicity of PoAstV in pigs.

Materials and Methods

A. Development of a differential multiplex real-time PCR that can be used to differentiate between members of the astrovirus family infecting pigs.

Available nucleotide sequences of the five PAstV types were aligned using Clustal W within DNASTAR (Lasergene 8), and a pair of degenerate primers capable of detecting all five PAstV types was designed based on a conserved region located in the region of ORF1b, which covered a fragment of 183 bp. Five different TaqMan probes specific for each type were also designed (Xiao et al., 2013). Two multiplex quantitative real-time RT-PCR assays were established: the PAstV1-2 PCR contained probes for detecting PAstV1 and PAstV2, and the PAstV3-4-5 PCR used probes for detecting PAstV3–PAstV5. The real-time RT-PCRs were carried out in 96-well plates. Amplification reactions were performed using an ABI 7500 Fast Real-Time PCR System (Applied Biosystems) under universal conditions: 30 min at 50 °C and 10 min at 95 °C, followed by 45 cycles of 15 s at 95 °C and 1 min at 60 °C. A sample was considered negative if no cycle threshold (C_T) was detected during the 45 amplification cycles. PAstV genomic loads (g fecal sample)−1 were calculated by multiplying the individual results from the quantitative real-time RT-PCR by 1667 [50 µl RNA preparation eluted from 50 µl fecal suspension×80 (4 ml PBS per 50 µl elution buffer)×2.5 (1 g per 0.4 g feces) per 6 µl PCR input].

B. Screening the U.S. pig population for prevalence of PoAstV pigs with and without enteric disease.

A total of 509 fecal samples were chosen randomly from routine diagnostic cases submitted during 2011–2012 to the ISU-VDL. Most of the samples (488; 95.9%) came from pigs with a history of diarrhea. The remaining 21 samples (4.1%) were submitted from farms with no history of diarrhea, but the pigs were suffering from other clinical signs such as respiratory or systemic disease. These samples originated on 255 farms located in 19 US states – Colorado, Iowa, Illinois, Indiana, Michigan, Minnesota, Missouri, North Carolina, North Dakota, Nebraska, Ohio, Oklahoma, Pennsylvania, South Dakota, Texas, Utah, Virginia, Wisconsin and Wyoming – with the age of the pigs ranging from suckling to adult pigs, including some samples from pigs of unknown age. The majority of submissions (53.4%; 272/509) came from Iowa due to the geographical location of the ISU-VDL. Fecal samples of ~0.4 g were re-suspended in 4 ml PBS, vigorously vortexed and centrifuged at 1500 g for 10 min. Viral RNA extraction was carried out on 50 µl fecal supernatant using a 5×MagMAX 96 Viral Isolation kit (Ambion) according to the manufacturer's instructions on an automated extraction platform (KingFisher Flex; Thermo Fisher Scientific). The extracted RNA was stored at −80 °C until use.

C. Isolation of selected PoAstV

Virus isolation on selected PCR positive samples was conducted on PK-15 cells as described (Shimizu et al., 1990). We also attempted virus propagation in colostrum-deprived pigs as we routinely do for swine Hepatitis E virus (Halbur *et al.*, 2001).

D. Experimental infection of pigs with diverse PoAstV

Three colostrum-deprived pigs from a high health herd negative for known enteric swine pathogens based on repeated PCR and no clinical history of diarrhea were utilized.

Results

Establishment of real-time PCR assays

Two newly established multiplex PAstV real-time RT-PCR assays were used on tenfold serial dilutions of PAstV plasmid standards. A detection limit of 5 genome equivalents (GEs) occurred around a CT of 37 in the PAstV1-2 multiplex assay, and a detection limit of 10 GEs occurred around a CT of 38–39 in the PAstV3-4-5 assay. Determined slopes, R2 and intercept values of the standard curves were −3.74, 0.998 and 40.6 for PAstV1, −3.77, 0.998 and 40.1 for PAstV2, −3.58, 0.998 and 43.4 for PAstV3, −3.63, 0.999 and 42 for PAstV4, and −3.78, 0.999 and 42.2 for PAstV5, respectively. The specificity of the probes was confirmed by blast analysis and by testing samples positive for other RNA viruses available in the laboratory, including porcine reproductive and respiratory syndrome virus and swine hepatitis E virus. No cross-amplification was observed between the PAstV types with the plasmids of each type or with other RNA viruses. This indicated that the two multiplex real-time RT-PCR assays allowed specific, efficient and sensitive detection of different PAstV types down to 10 GEs or less per reaction.

High PAstV prevalence in US pigs and infection of individual pigs with multiple PAstV types

PAstVs were revealed to have a high overall prevalence of 64.0% (326/509) in faecal samples, with the prevalence of PAstV1–PAstV5 ranging from 1.2 to 62.3%. High rates of co-infection (13.9%, 71/509) were also observed. Overall, one or more PAstVs were identified in 31.6% (25/79) of the suckling pigs, in 75.1% (136/181) of the nursery pigs, in 72.3% (107/148) of the grow-finish pigs and in 28.6% (2/7) of the mature pigs. The highest PAstV prevalence was found in nursery pigs, which was significantly ($P<0.05$) higher compared with suckling and mature pigs but was not significantly different from grow-finish pigs. Among the PAstV types, PAstV4 had the highest overall prevalence of 62.3%, with prevalence rates of 26.6% in suckling pigs, 74.0% in nursery pigs, 72.3% in grow-finish pigs and 28.6% in adult pigs. The highest numbers of genomic copies in individual pigs were 3.56×10^6 for PAstV1 (suckling pig), 6.05×10^6 for PAstV2 (suckling pig), 3.34×10^5 for PAstV3 (suckling pig), 1.80×10^9 for PAstV4 (suckling pig) and 5.46×10^8 for PAstV5 (nursery pig). In general, higher viral loads in PCR-positive pigs were identified in suckling pigs (PAstV1 and PAstV2) or nursery pigs (PAstV3–PAstV5); however, significant differences were only seen for PAstV4.

Sequencing analysis

Some of the real-time RT-PCR-positive samples were randomly selected for genome sequencing. The entire genomes of PAstV2-IA122, PAstV3-MO123 (Xiao et al., 2011), PAstV4-IL135 and PAstV5-IA122 were 6305, 6430, 6635 and 6480 bp excluding the poly(A) tail, respectively. Partial genome sequences of PAstV4-P2011-1 (2678 bp) and PAstV5-P2011-2 (2490 bp) including the 3′ end of the ORF1b gene, the complete capsid gene (ORF2) and the 3′-end untranslated regions (UTRs), and three fragments of PAstV1 (183 bp) were also obtained.

Relationship between PAstV

Phylogenetic analyses were carried out with the complete nucleotide and amino acid sequences of ORF2, and the complete/near-complete genome sequences of the PAstVs obtained in the present study and those available in GenBank, together with selected AstV sequences from other species (Fig. 1). Five clades corresponding to the five PAstV types were clearly delineated, with high bootstrap support similar to those described previously (Laurin et al., 2011; Shan et al., 2011). PAstV3-MO123 clustered with the only PAstV3 strain 16-2 described previously in Canada (Luo et al., 2011) and, notably, showed a close relationship with mink AstV, sea lion AstV, sheep AstV and human AstV strains suggesting past cross-species transmission and the same ancestral origin of the viruses. PAstV4-IL135 clustered with PAstV strain 35 (Shan et al., 2011). PAstV5-IA122 and PAstV5-P2011-2 clustered with strain 33 reported previously (Shan et al., 2011) but more distantly with the PAstV5 strain CC12 from Canada (Laurin et al., 2011), also indicating that there are sublineages within PAstV5.

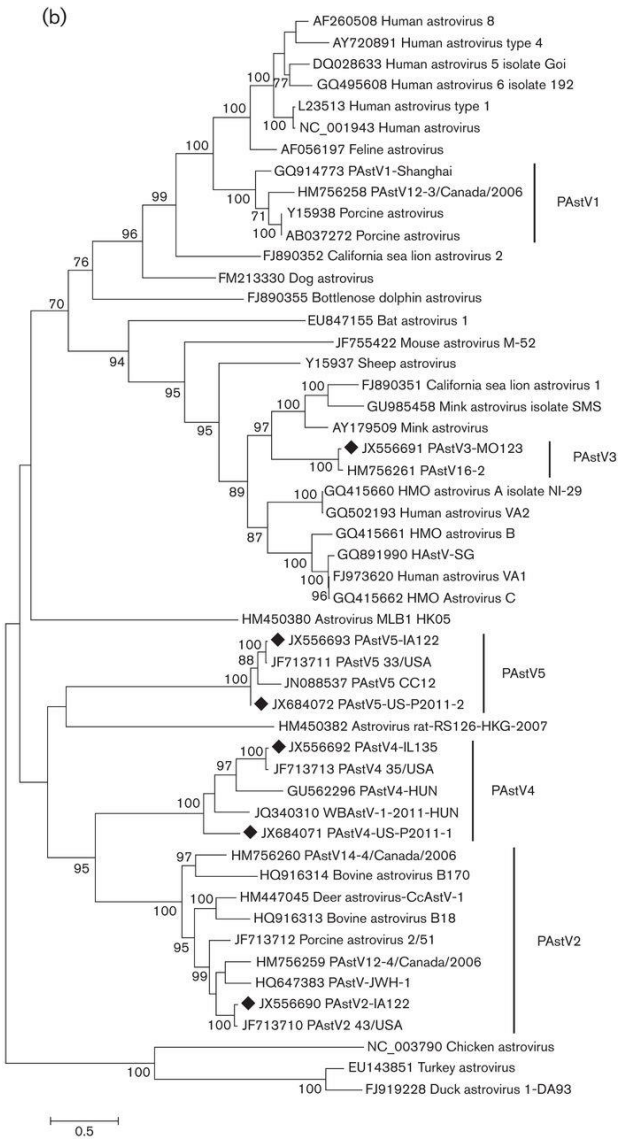

Fig. 1. Evolutionary tree based on the complete amino acid sequences of the ORF2 of 51 nucleotide sequences of AstVs.

PAstV Isolation

Fecal samples were selected for virus isolation from routine submission based on low Ct values as determined by quantitative real-time PCR. The results are summarized in Table 1.

Table 1. PAstV4 and PAstV5 isolation in PK-15 cells on selected PCR positive samples. Numbers indicate PAstV PCR Ct values.

	PAstV4			PAStV5		
Lab ID	P1	P2	P3	P1	P2	P3
141	20.1	Neg		Neg		
102	20.1	Neg		39.6	Neg	
316	20.3	Neg	Neg	27.2	33.6	Neg
148	20.3			Neg		
319	21.3	Neg		Neg	Neg	
147	21.6			Neg		
366	21.8	Neg		Neg	Neg	
378	21.8	Neg		38.7	Neg	
81	22.4	Neg		Neg		
94	22.6	Neg		Neg		
350	22.7	Neg		Neg		
184	22.8	Neg		Neg		
154	22.9	Neg		Neg	Neg	
135	23.2	Neg		Neg		
137	23.3	Neg		Neg		
157	23.3	Neg		Neg		
110	23.4	Neg		Neg		
103	23.4	Neg		Neg		
199	23.5	Neg		Neg		
97	23.6	Neg	Neg	34.6	37.1	Neg
198	23.7	Neg		Neg		
370	23.8	Neg		Neg	Neg	
322	24.1	Neg		Neg		
143	24.2	Neg		Neg		
96	24.4	Neg		36.2	Neg	
207	24.5	Neg		Neg		
217	24.8	Neg		Neg		
144	24.8	Neg		Neg		
11	25.1	Neg		Neg		
132	25.1	Neg		Neg		
150	25.4	Neg		Neg	Neg	
159	26.5	Neg		Neg	Neg	
93	27.6	Neg		24.3	Neg	
82	31.9	Neg		Neg	Neg	
98	32.0	Neg		Neg	Neg	
214	33.2	Neg		Neg	Neg	
319	Neg			Neg	Neg	Neg
366	Neg			Neg	Neg	Neg
35	Neg			Neg	Neg	Neg
123	Neg	Neg	Neg	Neg		
220	Neg			Neg		
239	Neg			Neg		

Infectious clone production

PAstV3 and PAstV4 clones were produced and tested in cell lines for infectivity. It has been reported that the introduction of ribozyme elements at both termini of the viral genomic cDNA in a DNA-launched (plasmid DNA transfection-based) reverse genetics system can improve the rescue efficacy of virus approximately 10–50-fold higher than the *in vitro*-transcribed RNA-based system and the traditional DNA-launched system without the engineered ribozyme elements (Huang *et al.* 2009). For comparison and to obtain a better rescue system for PAstV, we adopted both the traditional DNA-launched system without the engineered ribozyme elements and the new DNA-launched system with the engineered ribozyme elements reverse genetic system to

rescue PAstV3 and PAstV4. The full-length genomic cDNA of PAstV3 and PAstV4 were obtained and then sequentially cloned by two overlapping cDNA fragments into the pCI-neo mammalian expression vector, which carries the human cytomegalovirus (CMV) immediate-early enhancer/promoter region to promote constitutive expression of cloned DNA inserts in mammalian cells. Moreover, a hammer head ribozyme (HHRz) element and a hepatitis delta virus ribozyme (HDVRz) element were introduced at the 5' and 3'-terminus of both the PAstV3 and the PAstV4 genomic cDNAs, respectively, and the traditional D-launched method was also carried out in parallel. The recombinant plasmids were then sequenced and confirmed. As PAstV1 is reported to grow in the PK15 (Indik *et al*.2006), we transfected the recombinant plasmids of pCI-PAstV3-RZ and pCI-PAstV4-RZ (with ribozyme elements in boths ends) and pCI-PAstV3 and pCI-PAstV4 (without ribozyme elements in boths ends) into PK15 with Lipofectamine 2000 reagent (Invitrogen), and, for comparison, the IPEC-J2 cells (porcine intestinal columnar epithelial cells isolated from neonatal piglet mid-jejunum) and ST cells were also. The cells were frozen four days after transfection, and then were submitted to three freeze-thaw cycles, and the virus suspension was then used for subsequent passage through the routine way with 2 ug/ml of trypsin added into the medium without serum. The quantity of the expressed viral RNA in the transfected cells was checked by the real-time PCR we developed recently. The results shows that PAstV4 can be detected during the first 5 passages in ST cells and 3 passages in IPEC-J2 and PK15 cells, while PAstV3 can only be detected in the first passages of these cells, which may indicate these cells are not permissive for growing of the PAstV3 and PAstV4.

To further confirm the viral virulence *in vivo*, 400 ug of the recombinant plasmids, including 2 ml of cell culture inoculum from first passage of the viruses were then inoculated into CDCD pigs. However, none of the pigs seroconverted or developed PAstV viremia or shedding.

IDiscussion

Previous reports have revealed variable PAstV prevalence rates ranging from 39.1% in Japan to 80% in Canada based on serological and virological surveillance, respectively (Luo et al., 2011; Shimizu et al., 1990). In the present study, we investigated the prevalence of the different PAstV types in 509 pigs, of which 95.9% came from 255 farms with a history of diarrhea located in 19 U.S. states, and found that all five PAstV types are circulating in the USA. We also found the presence of two or more PAstV types in the same pig in 13.9% of the cases. A high overall prevalence of 63.9% (325/509) of PAstVs was detected, with 75.1% positive nursery pigs followed by grow-finish pigs (72.3%), suckling pigs (31.6%) and mature pigs (28.6%). The low prevalence and viral loads in mature pigs may indicate an age-restricting pattern for infection with PAstV; however, further investigations are needed to confirm associations with age, PAstV load and clinical performance.

Remarkably, **97.2% (317/326) of PAstV-positive pigs were infected with PAstV4, indicating the dominance of PAstV-4**. This is consistent with findings of a recent study in which 56 sequences obtained from the AstV-positive fecal samples were all confirmed to be PAstV4 (Mor et al., 2012). Moreover, in this study, besides the high prevalence of PAstV4, relatively low overall positive rates of PAstV1 (6.3%), PAstV2 (5.5%), PAstV3 (1.2%) and PAstV5 (5.3%) were found, indicating a remarkable diversity of PAstVs co-circulating in pigs in the USA. Notably, the highest prevalence rates of PAstV1, PAstV2 (except for grow-finish pigs where one of seven samples was positive), PAstV4 and PAstV5 were detected in nursery pigs, followed by grow-finish pigs for PAstV1 and PAstV4, and suckling pigs for PAstV2 and PAstV5. The highest prevalence of PAstV3 was found in suckling pigs, with only one additional positive sample identified in nursery pigs. Among pigs with no clinical history of diarrhea, the prevalence rates were 4.8% (1/21) for PAstV1, 0% (0/21) for PAstV2 and PAstV3, 71.4% (15/21) for PAstV4 and 4.8% (1/21) for PAstV5. Due to the low numbers of pigs without clinical history of diarrhea included in this study, and because the pigs were submitted for other clinical signs and therefore could not be considered to be healthy, statistical analysis between 'diarrhea' and 'non-diarrhea' pigs was not attempted.

Virus isolation from field samples and infection of pigs with infectious clones was not successful.

Reference List:

Blomstrom, A.L., Widen, F., Hammer, A.S., Belak, S., Berg, M., 2010, Detection of a novel astrovirus in brain tissue of mink suffering from shaking mink syndrome by use of viral metagenomics. J Clin Microbiol 48, 4392-4396.

Bridger, J.C., 1980, Detection by electron microscopy of caliciviruses, astroviruses and rotavirus-like particles in the faeces of piglets with diarrhoea. Vet Rec 107, 532-533.

De Benedictis, P., Schultz-Cherry, S., Burnham, A., Cattoli, G., 2011, Astrovirus infections in humans and animals - Molecular biology, genetic diversity, and interspecies transmissions. Infect Genet Evol 11, 1529-1544.

Fu, Y., Pan, M., Wang, X., Xu, Y., Xie, X., Knowles, N.J., Yang, H., Zhang, D., 2009, Complete sequence of a duck astrovirus associated with fatal hepatitis in ducklings. J Gen Virol 90, 1104-1108.

Gough, R.E., Collins, M.S., Borland, E., Keymer, L.F., 1984, Astrovirus-like particles associated with hepatitis in ducklings. Vet Rec 114, 279.

Huang, Y.W., Fang,Y., Meng,X.J., 2009. Identification and characterization of a porcine monocytic cell line supporting porcine reproductive and respiratory syndrome virus(PRRSV) replication and progeny virion production by using an improved DNA-launched PRRSV reverse genetics system.Virus Res.145, 1–8.

Imada, T., Yamaguchi, S., Mase, M., Tsukamoto, K., Kubo, M., Morooka, A., 2000, Avian nephritis virus (ANV) as a new member of the family Astroviridae and construction of infectious ANV cDNA. J Virol 74, 8487-8493.

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.

Jonassen, C.M., Jonassen, T.O., Saif, Y.M., Snodgrass, D.R., Ushijima, H., Shimizu, M., Grinde, B., 2001, Comparison of capsid sequences from human and animal astroviruses. J Gen Virol 82, 1061-1067.

Lan, D., Ji, W., Shan, T., Cui, L., Yang, Z., Yuan, C., Hua, X., 2011, Molecular characterization of a porcine astrovirus strain in China. Arch Virol 156, 1869-1875.

Laurin, M.A., Dastor, M., L'Homme, Y., 2011, Detection and genetic characterization of a novel pig astrovirus: relationship to other astroviruses. Arch Virol.

Luo, Z., Roi, S., Dastor, M., Gallice, E., Laurin, M.A., L'Homme, Y., 2011, Multiple novel and prevalent astroviruses in pigs. Vet Microbiol 149, 316-323.

Mendez, E., Arias, C. 2007. Astroviruses. In Fields virology, Knipe DM, H., PM, eds. (Philadelphia, Lippincott Williams & Wilkins), pp. 981-1000.

Mor S. K., Chander Y., Marthaler D., Patnayak D. P., Goyal S. M. (2012). Detection and molecular characterization of porcine astrovirus strains associated with swine diarrhea. J Vet Diagn Invest 24, 1064–1067.

Moser, L.A., Schultz-Cherry, S., 2005, Pathogenesis of astrovirus infection. Viral Immunol 18, 4-10.

Reuter, G., Pankovics, P., Boros, A., 2011, Identification of a novel astrovirus in a domestic pig in Hungary. Arch Virol 156, 125-128.

Shan T., Li L., Simmonds P., Wang C., Moeser A., Delwart E. (2011). The fecal virome of pigs on a high-density farm. J Virol 85, 11697–11708.

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.

Wang, Q.H., Kakizawa, J., Wen, L.Y., Shimizu, M., Nishio, O., Fang, Z.Y., Ushijima, H., 2001, Genetic analysis of the capsid region of astroviruses. J Med Virol 64, 245-255.

Xiao, C.T., Halbur, P.G., Opriessnig, T. 2012. Complete genome sequence of a newly identified porcine astrovirus genotype 3 strain US-MO123. J Virol. 86, 13126.

Xiao, C.T., Giménez-Lirola, L.G., Gerber, P.F., Jiang, Y.H., Halbur, P.G., Opriessnig, T., 2013. Identification and characterization of novel porcine astroviruses (PAstVs) with high prevalence and frequent co-infection of individual pigs with multiple PAstV types. J Gen Virol. 94, 570-582.