PORCINE ASTROVIRUS

SUMMARY

Etiology

- Porcine astrovirus (PAstV) is a non-enveloped RNA virus in the family *Astroviridae*.
- There are five known lineages with great genetic variability.

Cleaning and Disinfection

- Astrovirus (AstV) is resistant to heat and requires very acidic pH for inactivation; it can survive for a long time in the environment.
- There is little information on specific disinfectants for AstVs. Non-enveloped viruses are typically susceptible to aldehydes and halogens (bleach).

Epidemiology

- AstVs affect a wide variety of species. PAstV was first isolated from pigs in 1980.
- The zoonotic potential of AstVs is unclear; porcine-human AstV recombinants have been documented and human-to-pig transmission is suspected.
- PAstV has been isolated from pigs worldwide. Human AstV infections are most common in the winter, but there is no information about season incidence of AstV infection in pigs.
- Prevalence in swine can be very high; however, viral co-infection with other enteropathogens (e.g., rotavirus, transmissible gastroenteritis virus, porcine circovirus-2, and porcine hemagglutinating encephalitis virus) is often observed.

Transmission

- Transmission of PAstV is thought to be fecal-oral.

Infection in Swine/Pathogenesis

- PAstV is thought to cause mild, self-limiting secretory diarrhea. Young pigs are most affected. Viral co-infections may contribute to observed clinical signs.

Diagnosis

- AstV is difficult to propagate. Reverse transcription polymerase chain reaction (RT-PCR) assays are used to detect viral RNA. Immunohistochemistry and indirect immunofluorescence have both successfully detected AstV antigens.

- Enzyme linked immunosorbent assay (ELISA) and serum neutralization have been used to detect antibodies for AstV in pigs.

Immunity
- There are currently no available vaccines against AstV in any species.

Prevention and Control
- Cleaning and disinfection of affected facilities is critical to prevent enteric disease in swine.
- Standard biosecurity practices should also be in place.

Gaps in Preparedness
- Little is known about PAstV and its role as a swine pathogen. Although no zoonotic cases have been reported, the potential for cross-species transmission exists. Vaccine development may be beneficial to both the human and animal medical communities.
- To prevent and control outbreaks, more information is needed on cleaning and disinfection protocols.

OVERVIEW

Porcine astrovirus (PAstV) is a non-enveloped RNA virus belonging to the family *Astroviridae*. There are at least five distinct lineages (PAstV1–PAstV5), all of which have been known to circulate in swine herds in the United States. Isolates from a single herd of pigs have shown high genetic diversity and variability. Virulence appears to vary by serotype.

Astrovirus (AstV) has a very wide range of hosts but infection is generally species-specific. PAstV is found worldwide with variable prevalence among herds. In swine, the virus causes a mild, self-limiting secretory diarrhea predominantly in piglets and weanlings; however, it has also been isolated from healthy adult pigs. In humans, astrovirus is the second leading cause of infantile gastroenteritis in children after rotavirus.

Though difficult to isolate, AstV has been successfully propagated in porcine kidney cells. Fecal samples are the primary source for virus isolation, although there are reports of extraintestinal AstV isolation from pigs, birds, humans, and mink. Reverse transcription polymerase chain reaction (RT-PCR) assays are used to detect viral RNA. Immunohistochemistry and indirect immunofluorescence have both successfully detected AstV antigens. Enzyme linked immunosorbent assay (ELISA) and serum neutralization have been used to detect antibodies for AstV in pigs.

There are currently no available vaccines against AstV in any species. Promising polyclonal antibody titers have been shown in rabbits and chickens inoculated with a baculovirus-produced chicken AstV (CAstV) capsid protein vaccine. Whether or not the antibodies are protective remains to be seen. The development of this antigen has been utilized in diagnostic tests and vaccine trials, but further work is required.

AstV is a major concern in human infants, but no zoonotic cases have been reported to date. Its ability to rapidly mutate, and the ability of an animal to become co-infected with two different strains, sets the stage for a recombination event from which a zoonotic strain could emerge. AstV is a public health concern in humans as it has been implicated in foodborne illnesses and can survive in ground water. Further research and investigation into the pathogenesis of AstV and vaccine development would benefit both veterinary and human medicine.

LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics
Porcine astrovirus (PAstV) is a small, non-enveloped, single stranded positive sense RNA virus named for its 5–6 pointed star-like configuration that can be seen via negative electron microscopy.[1] Astrovirus (AstV) belongs to the family *Astroviridae*, which is divided into two genera, *Mamastrovirus* and *Avastrovirus*. These genera affect mammals and avian species respectively.[1]

1.2 Strain Variability
The genome of AstV is composed of three open reading frames that encode for nonstructural proteins (ORF1a), RNA-dependent RNA polymerase (ORF1b), and capsid proteins (ORF2) respectively.[5] An untranslated region (UTR) is present at the 5'end. The 3'end contains both a UTR and a poly-A tail.[7] There is a highly conserved nucleotide sequence among PAstV at the 5'end of ORF2 which is useful for genomic analysis and PCR detection.[3]

A study from Canada found a highly conserved nucleotide sequence within the RNA-dependent RNA polymerase gene located between ORF1b and ORF2 in the genome of the *Astroviridae* family.[3] Analysis further grouped PAstV into 3 subgroups or clusters: Group I strains were closely related to PAstV 1, human AstV, and feline AstV strains; Group II strains had little homology and lacked the nucleotide sequences between ORF 1b and ORF2 that is highly conserved among the other PAstV; Group III strains were related to mink, ovine, and some newly discovered human AstV strains.[3] This same study showed no evidence of recombination in AstV of swine.[3] However, a Chinese study published in 2011 showed that one particular PAstV strain, JWH-1, was closely associated with a novel AstV of deer.[9] The diversity of PAstV is still further supported by data showing that strains collected from swine on a single farm and organized into subgroups seem to have relatively low homology.[2]

Within PAstV, there are at least five distinct lineages (PAstV1–PAstV5), each of which have been known to circulate in herds in the United States.[8] Based on phylogenetic analysis, each of the five lineages may have an independent origin, with vast genetic variability evidenced by greater homology between strains of different lineages than within strains of a single lineage.[4]

2. Cleaning and Disinfection
2.1 Survival
AstV remains stable and infectious within groundwater.[10] AstV persists in tap water at 4°C for 45 days with only a 1.2 log titer reduction (LTR).[11]

2.2 Disinfection
Experimentally, PAstV resisted lipid solvents and remained stable when exposed to temperatures of 50°C for 30 minutes.[1] PAstV also retained its infectivity at pH 4.0 for 3 hours, but exposure to pH 3.0 for 3 hours decreased infectivity two-fold.[1] Infectivity of AstV when exposed to chlorine displayed 2.5 LTR after 1 hour contact time.[11]

3. Epidemiology

3.1 Species Affected
The family *Astroviridae* contains two genera: *Mamastrovirinae* consisting of six mammalian species-specific viruses, and *Avastrovirinae*, consisting of three avian species-specific viruses. AstV belonging to

Avastrovirinae cause more severe disease in their host species than do any of the *Mamastrovirinae* viruses.[3,12]

AstV was first detected in feces of human infants, and later a cytopathic porcine strain was isolated from diarrheic feces of swine in 1980.[1] AstV has also been isolated from mink[6], sheep, cattle[14], deer[9], dogs, cats, wild boars[15], chickens[16], ducks[17], turkeys[18], bats[3,19], and marine mammals.[20]

3.2 Zoonotic Potential

It was previously accepted that AstVs were host restricted and species-specific, but phylogenetic analysis has suggested the possibility of interspecies recombination.[18] This evidence, coupled with the diverse array of species affected and the genetic diversity within *Astroviridae,* makes interspecies transmission with virus adaption a possibility.[7] Co-infections of a single animal with multiple AstV strains has been reported.[8]

In one study, humans occupationally exposed to turkeys were found to seroconvert to Turkey AstV-2 (TAstV).[6] A mammalian-like AstV was detected in the avian European Roller species for the first time in China in 2015.[21] Phylogenetic analysis of Canadian isolates showed that PAstV-1, PAstV-2, and PAstV-3 were more closely related to human AstVs and AstVs from other animals than to each other.[8]

Emergence of porcine-human AstV recombinants has occurred in areas where pigs and humans live in close proximity with frequent interaction.[7] Viral transmission from humans to pigs is suspected while the reverse has not been described.[7]

A recombination event may have occurred between a human and sea lion isolate, suggesting either a human, a sea lion, or a third host was infected with both isolates simultaneously, resulting in emergence of a new strain.[20]

3.3 Geographic Distribution

PAstV has been isolated from pigs worldwide irrespective of age, season, or climate. PAstVs have been found in South Africa, Czech Republic, Hungary, Canada[3], Columbia[7], Croatia[4], United States[8], South Korea[22] and China[9]. Distribution of each lineage is variable. PAstV-1 is predominant in Japan while PAstV-4 predominates in Hungary, South Korea, and the United States. PAstV-1, -2, and -3 are present in Canada.[15] In 2014, Croatia reported the presence of PAstV-3 viruses in a herd, the first time PAstV-3 has been detected in Europe.[4]

Human AstV seem to have the highest incidence of infection during winter months in temperate climates, but infections have also been reported in spring and summer months.[7] No studies in swine have been published regarding seasonal incidence of PAstV.

3.4 Morbidity and Mortality

Prevalence of PAstV on a single farm is variable and may be geographically dependent. Diagnostic testing of diarrheic samples from throughout North America found PAstV in 61.7% of samples, 20% of which were positive for PAstV alone while the remainder of positive samples contained PAstV and another known diarrhea-causing virus (such as rotavirus, transmissible gastroenteritis virus, porcine circovirus-2, and porcine hemagglutinating encephalitis virus).[2] A report from Canada detected PAstV-positive RT-PCR results from 80% of healthy pigs at time of slaughter for market.[3] Fecal samples from a high density farm in North Carolina had PAstV present in 75% of samples taken from healthy and diarrheic piglets.[5] Seroconversion rates of swine herds have ranged as high as 83% in healthy adult pigs.[1]

Experimental AstV infection in 1 day old broiler chickens causes growth retardation.[23] No reports have indicated PAstV affects growth of pigs. Human AstVs are implicated in 20% of nonbacterial diarrhea in

elderly or immunocompromised individuals, and are one of the leading causes of diarrhea in human infants.[7] There have also been reports of extra-intestinal detection of AstV in pig, mink, and avian species making the virus' clinical manifest and pathogenesis even more obscure[4-6].

There is a possibility AstV may cause persistent infections in certain species. Persistent infections have been reported in insectivorous bats[28] and in a human child[13], and may be a reasonable explanation for AstV detection in fecal samples of a high percentage of apparently healthy adult pigs.[26]

4. Transmission

All published reports support the fecal-oral route in PAstV transmission. Cesarean-delivered, colostrum-deprived (CDCD) gnotobiotic piglets developed mild diarrhea five days post-inoculation with filtered feces from a diarrheic piglet.[12] Following euthanasia, PAstV presence was confirmed in intestinal tissues by electron microscopy.[12] CDCD pigs orally inoculated with a PAstV isolate also developed mild diarrhea, seroconverted, and shed infectious PAstV in their feces.[1][1]

5. Infection in Swine/Pathogenesis

The pathogenesis of PAstV-induced diarrhea is poorly understood, especially in non-human mammals, and was previously thought to localize to the intestine. Bovine AstV has been shown to be M-cell trophic throughout the intestine resulting in mild pathology even in the absence of clinical disease.[14] Recently, extra-intestinal AstV has been found in brain tissue of mink suffering shaking mink syndrome[6], from the brain of a human child suffering encephalitis[25], the liver in ducks with hepatitis[17], and the blood of pigs with positive PAstV fecal samples.[26] A study of TAstV-2 in young poults revealed mild histopathological changes of the intestines, lacking inflammatory lesions and cell death, despite severe diarrhea.[27] A proposed mechanism for this is an increase in TGF-B, a potent immunosuppressive cytokine.[27]A human AstV strain was found to experimentally induce diarrhea by disrupting tight junctions between intestinal epithelial cells, thus increasing permeability and access of the virus to underlying structures, including blood vessels.[24] Despite AstV being detected systemically, it is believed that viral replication is primarily limited to the absorptive epithelial cells of the intestinal tract.[27]

5.1 Clinical Signs

Pigs experimentally inoculated orally with PAstV develop a mild, self-limiting enteric disease characterized by secretory diarrhea.[1] Natural infections are more severe, likely due to viral co-infections contributing to clinical signs.[1]

Clinical signs vary among species. Renal and hepatic involvement accompany intense enteric disease in avian species.[7,17,21,27] AstV is suspected of causing shaking mink syndrome in mink, resulting in ataxia, abnormal gait, tremors, and other neurological signs.[6] Gnotobiotic calves experimentally inoculated orally with bovine AstV showed no clinical signs.[14]

5.2 Post Mortem Lesions

Studies investigating the anatomic pathology of PAstV are limited, likely due to the lack of PAstV-caused mortality. Minimal gross changes have been seen upon necropsy of PAstV-infected pigs while histopathological alterations have not been investigaed.[12]

There have been investigations into the pathologic changes in turkey poults experimentally infected with TAstV-2. Upon post mortem examination, the intestines were dilated, 3–5 times normal size, and distended with gas and fluid.[27] Despite severe diarrhea, histopathology was unremarkable; observed changes included mild villous atrophy, very mild crypt hyperplasia, and minimal mononuclear infiltrate.[27]

6. Diagnosis

6.1 Clinical History

PAstV should be suspected in any piglet with diarrhea.[7] The diarrhea is most likely the result of co-infection and other diarrhea causing viruses should be suspected.[2] Healthy adult pigs are less likely to show clinical signs when infected with PAstV. [1]

6.2 Tests to Detect Nucleic Acids, Virus, or Antigens

Virus isolation for PAstV has proven difficult. PAstV isolation and propagation requires the use of pig kidney cells with the presence of trypsin.[1] Because of this, direct electron microscopy is often used to confirm PAstV infections clinically.[12]

Reverse transcriptase polymerase chain reaction (RT-PCR) is utilized to diagnose and analyze AstVs. Primers used for detection have been developed for bats which targets the 3' end of the ORF1b gene, a highly conserved region of the *Astroviridae* genome which encodes for the RNA dependent RNA polymerase.[28] This primer has been used in many studies to detect any member of the *Mamastrovirus* genus.[28] Primer sets specific for porcine strains have also been described.[3]

Due to the limited knowledge of the pathogenesis of AstV and its poor growth in cell culture, development of virus specific antigen tests is minimal. A baculovirus-produced CAstV capsid protein inoculated into rabbits resulted in polyclonal antiserum production, which was shown to be useful in indirect immunofluorescence, immunohistochemistry, and virus neutralization assays.[16] Indirect immunofluorescence and immunohistochemistry have been successfully performed for PAstV.[1,16]

6.3 Tests to Detect Antibody

Enzyme linked immunosorbent assays (ELISAs) have been used for determining the rate of human seroconversion to TAstV.[16,18] Baculovirus-produced TAstV capsid protein was used as capture antigen while polyclonal anti-TAstV from capsid protein immunized rabbits was used for detection.[16,18] Similarly, baculovirus-expressed CAstV capsid protein was used as capture antigen in an ELISA to detect anti-CAstV antibody in chicken serum. [15] Serum neutralization has been used to accurately detect PAstV.[1]

6.4 Samples

6.4.1 Preferred Samples

PAstV has most successfully and consistently been isolated from the feces of affected animals.[5,29] More recently, PAstV has been isolated from the blood of pigs with PAstV-positive fecal samples.[26] Avian species appear to have more systemic involvement with AstV infections; virus has been isolated from thymus, Bursa of Fabricius, spleen, liver, and jejunum.[7]

*6.4.2 Oral Flu*ids

The suitability of oral fluids for PAstV diagnosis has not been evaluated.

7. Immunity

7.1 Post-exposure

Pigs experimentally infected with PAstV shed infectious virus in their feces seven days post infection and developed neutralizing antibody titers 14 days post infection.[3] Chickens, vaccinated with a baculovirus-expressed CAstV capsid protein fused to GST and administered with oil adjuvant, developed CAstV-specific antibody titers four weeks post-vaccination; however, chickens vaccinated with baculovirus-expressed CAstV capsid protein alone, in combination with oil adjuvant, did not develop virus-specific antibody until four weeks after a booster vaccination. In both experiments, the majority of chickens

produced CAstV neutralizing antibody.[16] Whether the anti-CAstV antibodies produced by these animals will protect against challenge remains to be determined.

7.2 Vaccines
There are currently no commercially available PAstV vaccines. Chickens immunized with a baculovirus-expressed CAstV capsid protein developed virus-specific antibody, and this technique of producing virus-like particles for vaccination shows great promise but is not currently available commerically.[16]

Antigenic diversity among PAstV strains in a given location[2] presents a challenge for vaccine development and disease prevention.

7.3 Cross-protection
Cross protection has not been observed in humans with HAstV-1 antibodies when exposed to TAstV-2.[18] The antibodies appear to be specific to their respective capsid proteins.[18] Little is known of the antigenic relationship among the many strains of AstV.

8. Prevention and Control
Due to the characteristics of AstV, its ubiquity, and its mutability, preventing AstV infections may prove to be difficult. As a hardy, non-enveloped virus that is resistant to heat and requires very acidic pH for inactivation, it can survive for a long time in the environment.[1] The best means to prevent infection would be immunization of sows allowing passive transfer of antibodies through colostrum to neonates, removal of diarrheic pigs from the herd, and implementation of greater sanitation standards within a facility.

9. World Organization for Animal Health Terrestrial Animal Health Code
PAstV is not covered in 2015 OIE Terrestrial Animal Health Code and there are currently no recommendations on trade restrictions.

10. Gaps in Preparedness
Porcine AstV causes a mild, self-limiting secretory diarrhea predominantly in piglets, but has been isolated from healthy adult pigs as well.[1] Despite being extremely common in a wide range of hosts, the pathogenesis of AstV is poorly understood. Further investigation is needed to inform producers about prevention and treatment.

Because of its hardiness, mutability, and wide host range, there is potential for AstV zoonotic infection although there have been no reports to date. Despite evaluations of a potential CAstV capsid protein antigen as a vaccine candidate in chickens, little research has been performed evaluating the use of vaccines in swine. Additional investigation would better prepare both the veterinary and medical community in the event of a zoonotic infection and the possibility of an outbreak.

ACKNOWLEDGEMENTS

To cite:
Proietto S, Killoran K, Leedom Larson KR. Porcine astrovirus. Swine Health Information Center and Center for Food Security and Public Health, 2016. http://www.cfsph.iastate.edu/pdf/shic-factsheet-porcine-astrovirus.

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