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Methods For Production Of The Porcine Astrovirus 4 Capsid Spike Antigen And Its Use In Serological Assays And Vaccines

Tech ID: 34323 / UC Case 2025-913-0

BACKGROUND

Porcine astrovirus (PoAstV) was first detected by electron microscopy in the feces of piglets with diarrhea in 1980. There are five known genotypes of PoAstV, which are thought to be more closely related to other species than to each other. This divergence among genotypes suggests different ancestral origin of PoAstVs. PoAstVs have been detected across the globe including South Africa, Canada, China, Colombia, and Chile. All five genotypes are present in the US with high incidence. It is currently thought to be endemic in commercial swine in the US and potentially elsewhere.

One study of fecal samples from 509 pigs from 255 farms across 19 US states showed PoAstV4 was the most prevalent genotype in 62% (317/509) of samples. At least one PoAstV genotype was found in 64% (326/509) of the samples. It is common for multiple astroviruses to be detected in a single pig at once, which could provide opportunity for recombination to occur and lead to the emergence of new strains.

Multiple studies have connected PoAstV to a range of disease manifestations, with the virus frequently detected in feces of pigs displaying diarrheal symptoms as well as asymptomatic pigs. PoAstV5 is a cause of clinical enteritis, while PoAstV3 has been identified and characterized in the central nervous system of pigs with neurologic signs and nonsuppurative polioencephalomyelitis. Additionally, PoAstV4 has been identified in nasal samples from pigs with respiratory disease.

Researchers have investigated cases of bronchitis and/or tracheitis in pigs where PCR results were negative for influenza virus and other known causes of respiratory virus infection. Next generation sequencing revealed reads of PoAstV4. In a retrospective study of cases of tracheitis and/or bronchitis of unknown etiology, RNA in situ hybridization (ISH) was used to detect PoAstV4 RNA in airway epithelium (trachea, bronchi, or bronchioles), revealing PoAstV4 RNA in 73% (85/117) of cases. So PoAstV4 is strongly associated with lesions of epitheliotropic viral infection in young pigs with clinical respiratory disease.

Accurate tests and vaccines for PoAstV4 infection are clearly needed.

TECHNOLOGY DESCRIPTION

Researchers in Rebecca Dubois's lab at UC Santa Cruz have isolated and determined the structure of the PoAstV4 capsid spike protein. The protein sequence of PoAstV4 capsid spike has low sequence identity to the capsid spikes of other astrovirus species and therefore the boundaries of the domain could not be determined by sequence alignment alone. Instead, the spike domain boundaries were determined by predicting the structure of the full PoAstV4 capsid

Permalink

CONTACT

Jeff M. Jackson jjackso6@ucsc.edu



INVENTORS

DuBois, Rebecca M.

OTHER INFORMATION

KEYWORDS

porcine, pigs, astrovirus, PoAstV,
PoAstV4, respiratory infection, GI
infection, viral, ELISA, vaccine

CATEGORIZED AS

- Veterinary
 - Diagnostics
 - Large Animal
 - Vaccines

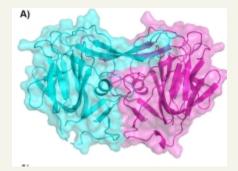
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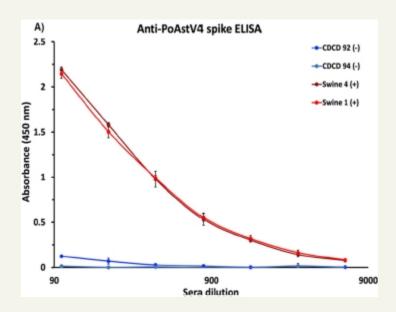
protein using AlphaFold2.

A PoAstV4 spike construct containing residues 420 to 655 was designed rom the full-length capsid protein of PoAstV4. The recombinant PoAstV4 spike protein expressed in *E. coli* resulted in high yields of soluble protein (>12 mg / Liter E. coli). The calculated molecular weight of the PoAstV4 spike is 27.8 kD (+1.6 kD cleavable histidine affinity tag). This was confirmed using SDS-PAGE analysis of purified PoAstV4 spike. Size exclusion chromatography showed purified PoAstV4 spike protein eluted at the approximate size of a homodimer, which suggests that the recombinant protein is folded correctly.

The PoAstV4 capsid spike crystal structure reveals a homodimeric protein formed of mainly beta-strands, similar to other animal and human astrovirus capsid spikes, and consistent with in-solution studies with size-exclusion chromatography (FIG. 2B). The structure confirms that the predicted PoAstV4 spike residues 420 to 655 form the structural domain. Interface analysis using the PDBePISA server reveals 4660 Å buried at the dimer interface, with 64-65 interacting residues in each chain.



An enzyme-linked immunosorbent assay (ELISA) was developed to deterimine if the recombinant PoAstV4 spike could be used to detect PoAstV antibodies. Serum samples collected during an outbreak of PoAstV4 in Iowa showed IgG reactivity towards the PoAstV4 spike antigen. Sera from presumed seronegative cesarean-derived, colostrum-deprived (CDCD) pigs, which do not receive maternal antibodies, exhibited minimal reactivity.



APPLICATIONS

- ▶ Serum assays for porcine astrovirus infection.
- ELISA
- Positive control
- Immunogenic agents for porcine astrovirus spike proteins
- For raising antibody reagents
- Polyclonal antisera

- Monoclonal antibodies
- For immunoprotection
- Vaccine development

ADVANTAGES

- ▶ Specific to the porcine astrovirus 4 genotype: a very common genotype of porcine astrovirus
- ▶ PoAstV4 causes both GI and respiratory symptoms

INTELLECTUAL PROPERTY INFORMATION

Patent Pending

RELATED MATERIALS

ADDITIONAL TECHNOLOGIES BY THESE INVENTORS

- ▶ Glycoengineering Of The Foldon Protein Trimerization Domain To Shield It From Antibody Immune Responses
- ► Human Astrovirus Neutralizing Monoclonal Antibody Sequences
- ▶ Simplified Workflow For Hybridoma Antibody Sequencing
- Methods To Rapidly Measure Antibodies And Other Biomolecules In Clinical Specimens Utilizing Biolayer Interferometry

University of California, Santa Cruz

Industry Alliances & Technology Commercialization

Kerr 413 / IATC,

Santa Cruz, CA 95064

Tel: 831.459.5415

innovation@ucsc.edu

https://officeofresearch.ucsc.edu/

Fax: 831.459.1658

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