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Porcine Astrovirus Type 3 in Central Nervous System of Swine with Polioencephalomyelitis

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Using next-generation sequencing, we identified and genetically characterized a porcine astrovirus type 3 strain found in tissues from the central nervous system of 1 piglet and 3 sows with neurologic signs and nonsuppurative polioencephalomyelitis. Further studies are needed to understand the potential for cross-species transmission and clinical impact.

Astroviruses have been identified in a variety of mammals and birds; infection is often asymptomatic (*1*). Recently astroviruses have been implicated in cases of encephalomyelitis in humans, mink, cattle, and sheep (*2–5*). We describe the use of unbiased next-generation sequencing to identify and genetically characterize a porcine astrovirus type 3 (PoAstV-3) in central nervous system (CNS) tissues of a 5-week-old piglet and 3 sows with neurologic signs and histopathologic lesions compatible with a neurotropic viral infection.

A multisite swine production farm submitted swine neurologic cases on 3 different occasions over a 9-month period to the Iowa State Veterinary Diagnostic Laboratory (Ames, Iowa, USA); 1 submission (2 live piglets) represented a population of 4–12-week-old pigs and 2 submissions (submission 2, two live sows; submission 3, head and tissue of sow) representing sows. In all cases, affected swine exhibited clinical signs that ranged from hind limb weakness to quadriplegia and occasionally convulsions (Video, https://wwwnc.cdc.gov/EID/article/23/12/17-0703-V1.htm). The sow farm reported a case-fatality rate of 100%. The young pigs, which were farrowed from sows from the aforementioned sow farm, originated

from 2 commercial grow-out facilities that reported a case-fatality rate of 75%. Histologic lesions in the CNS were consistent with a viral etiology. The following viruses were not detected in CNS samples by PCR: porcine reproductive and respiratory syndrome virus types 1 and 2, porcine circovirus 2, suid alphaherpesvirus 1, teschovirus A, sapelovirus A, or atypical porcine pestivirus. No pathogens were isolated by bacterial culture. Because of the persistence and severity of clinical signs, histologic lesions, and lack of detection of a viral etiology, two 5-week-old piglets and 4 sows with neurologic signs were submitted by a veterinarian for diagnostic

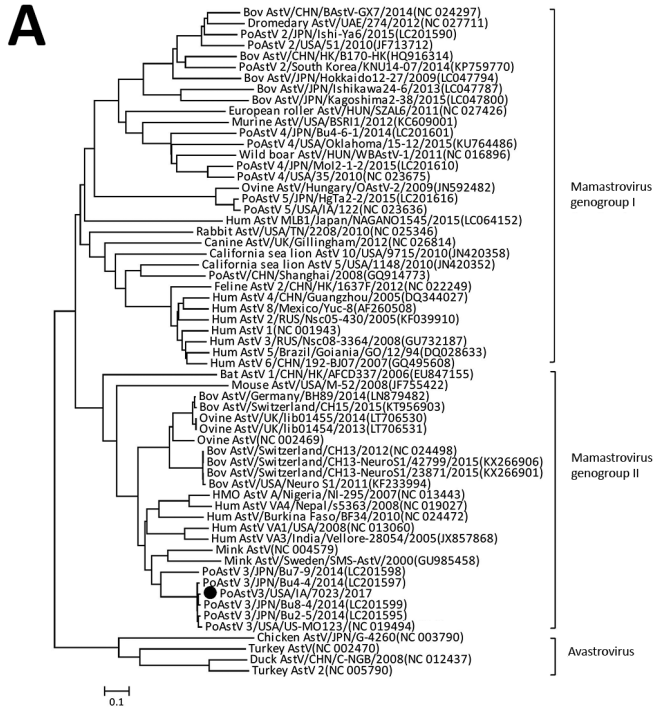

Figure. Phylogenetic trees of capsid protein (A), RNA-dependent RNA polymerase protein (B), and whole-genome nucleotide (C) sequences of a PoAstV type 3 strain (PoAstV3/USA/IA/7023/2017, filled circle) from central nervous system tissues of sows with neurologic signs and histopathologic lesions compatible with neurotropic viral infection compared with 66 reference viruses available in GenBank (accession numbers shown in parentheses), which came from multiple animal species (as indicated). We performed alignment using the Muscle model and constructed phylogenetic trees using the neighbor-joining method in MEGA6 (http://www.megasoftware.net). Virus types are labeled. AstV, astrovirus; PoAstV, porcine astrovirus. Scale bars indicate nucleotide substitutions per site.

testing by histopathology and next-generation sequencing. Histologic examination revealed severe, nonsuppurative polioencephalomyelitis in 3 of 4 sows and 1 of 2 piglets (online Technical Appendix Figure, https://wwwnc.cdc.gov/EID/article/23/12/17-0703-Techapp1.pdf).

We performed metagenomic sequencing for each animal using pooled RNA extracted from the cerebrum, cerebellum, brain stem, and spinal cord as previously described (*6*,*7*). We analyzed the sequences obtained using the MiSeq System (Illumina, San Diego, CA, USA) by using Kraken, an ultrafast and highly accurate program for assigning taxonomic labels by examining the k-mers within a read and querying a standard Kraken database with those k-mers (*8*). We assembled reads de novo using CLC Genomics Workbench (QIAGEN, Valencia, CA, USA) and identified the contigs by blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The largest contig, encompassing ≈2,000 reads, encoded a near-complete astrovirus genome of 6,461 nt and was designated PoAstV3/USA/IA/7023/2017 (GenBank accession no. KY940545). This sequence originated from a sow sample. A near-complete astrovirus genomic sequence was also obtained from the piglet (contig length 5,935 bp; E = 0) and had 100% nucleotide identity to PoAstV3/USA/IA/7023/2017. We also identified porcine endogenous retrovirus (contig lengths 1,865 bp and 1,317 bp; E = 0) in sow samples. When using a minimum contig length of 500 nt, we identified rocilivirus (contig length 832 bp; 32 reads; E = 0; GenBank accession no. KU058672.1) in piglet samples.

Phylogenetic comparisons of the capsid protein sequence, RNA-dependent RNA polymerase protein sequence, and whole-genome nucleotide sequence placed PoAstV3/USA/IA/7023/2017 in the same cluster as other strains of PoAstV-3 (Figure, panels A–C). The isolate we identified was most closely related to PoAstV3/USA/US-MO123 (GenBank accession no. NC_019494.1; 94.1% amino acid identity; online Technical Appendix Table 1), which was detected in a swine fecal sample (*9*). On the basis of these phylogenetic analyses, PoAstV3/USA/IA/7023/2017 is more closely related to neurotropic astroviruses from humans, minks, cows, and sheep (*2*–*5*) than to PoAstV-1, PoAstV-2, PoAstV-4, and PoAstV-5.

We detected viral RNA by using a PoAstV-3 quantitative real-time PCR with previously fresh-frozen CNS tissues from animals with polioencephalomyelitis (online Technical Appendix Table 2). We did not detect viral RNA in serum, feces, lung, liver, kidney, or spleen samples of animals with histologic lesions or any sample from animals without histologic lesions (*9*).

We describe the identification and genetic characterization of PoAstV-3 in CNS tissue from a piglet and sows with neurologic signs and histologic lesions compatible with a neurotropic virus similar to those described in neurotropic astrovirus cases in other species (*2*–*5*). In humans, disease is primarily associated with immunocompromised patients. In cows, the virus is not commonly detected in feces, and the disease does not appear to be associated with immunocompromised animals (*4*). In this case, PoAstV-3 was not detected in feces of affected animals, and evidence of immunosuppression was lacking. The overall PCR prevalence of PoAstV-3 in feces of pigs in North America is reported to be low (1.2%) (*10*).

The PoAstV-3 we identified had 92.2% nucleotide sequence similarity to PoAstV-3 identified from a survey that evaluated feces samples from pigs (*9*).The significance of this finding is unclear. Investigations are needed to clarify the ecology and epidemiology of PoAstV-3 and the pathophysiology of neurotropic astroviruses. Studies have demonstrated the potential for recombination between porcine and human astroviruses, suggesting zoonotic potential (*9*,*10*).

Dr. Arruda is an assistant professor and diagnostic pathologist at the Iowa State University Veterinary Diagnostic Laboratory. Her primary field of research is the infectious diseases of swine.

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Avian Influenza A(H7N9) Viruses Co-circulating among Chickens, Southern China

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During 2016–2017, three avian influenza A(H7N9) viruses were isolated from chickens in southern China. Each virus had different insertion points in the cleavage site of the hemagglutinin protein compared to the first identified H7N9 virus. We determined that these viruses were double or triple reassortant viruses.

Since its first documentation on March 30, 2013, through March 16, 2017, avian influenza A(H7N9) virus has caused 5 epidemic waves of infection among humans in China, resulting in 1,307 laboratory-confirmed clinical cases and 489 deaths (*1*). According to reports of H7N9 virus outbreaks among humans in China, the virus clustered into the Yangtze River Delta lineage and the Pearl River Delta lineage (*2*). As with most low-pathogenicity avian influenza viruses, the early H7N9 avian influenza virus produced mild symptoms in domestic poultry and was therefore generally only detected through active virologic surveillance (*3,4*).

[1]These authors contributed equally to this article.

In April 2017, H7N9 viruses (isolates A/chicken/Guangdong/Q1/2016, A/chicken/Guangdong/Q26/2017, and A/chicken/Guangdong/Q39/2017, hereafter Q1, Q26, and Q39) were identified from lung samples of chickens that were collected from Guangdong, China, in June 2016 and January 2017. We sequenced all 8 genes of these viruses to trace the origin and clarify the genetic properties. The nucleotide sequences are available from GenBank (accession nos. MF280181–204).

The H7 hemagglutinin (HA) gene of all 3 viruses belonged to the Yangtze River Delta lineage A (Figure). However, unlike the early H7N9 virus, the HA genes were 1,695 bp and coded 565 aa, and the isolates had 4 inserted amino acids at cleavage sites (KRTAR¯G). In addition, Q26 and Q39 had 4 continuous basic amino acids at cleavage sites (KRKRTAR¯G), which is a characteristic of highly pathogenic avian influenza virus (Online Technical Appendix, Table 1, https://wwwnc.cdc.gov/EID/article/23/12/17-0782-Techapp1.pdf). Q1 had a mutation (Q226L) at the receptor binding site of the HA protein, indicating a higher binding affinity for sialic acid α2,6, a characteristic of human cell-surface receptors (*5*).

Both Q1 and Q39 had an NA gene of Yangtze River Delta lineage A, whereas the NA gene of Q26 was of Pearl River Delta lineage (online Technical Appendix Table 1, Figure). A246T and R292K, which are related to drug resistance, had no substitution in the NA protein of the viruses we analyzed.

The polymerase basic (PB) 1 and 2, polymerase acidic, and nonstructural genes of Q1 and Q39 were all of Yangtze River Delta lineage A, and nucleoprotein genes were of Yangtze River Delta lineage B. The PB2 and nucleoprotein genes of Q26 were of Yangtze River Delta lineage A; PB1, polymerase acidic, and nonstructural genes of Q26 were clustered to the Pearl River Delta lineage (online Technical Appendix Table 1, Figure). E627K and D701N had no substitution in the PB2 protein of the viruses, which was thought to contribute to the adaptation, replication, and virulence of influenza viruses in humans and mice (*6*,*7*).

Of particular note, the matrix M gene of Q1 clustered into A/goose/Guangdong/1/96-lineage (H5N1) (GSGD96 lineage) and had a nucleotide of 94.8%. However, the matrix genes of Q26 and Q39 clustered into Yangtze River Delta lineage B of H7N9 virus (online Technical Appendix Table 1, Figure).

To clarify the pathogenicity and transmission of the virus, we inoculated 11 chickens with each isolate (10^6 50% egg infectious dose [EID_{50}] in 0.1 mL of phosphate-buffered saline) and 3 chickens with 0.1 mL phosphate-buffered saline as the control group. We observed all chickens for clinical symptoms for 14 days. The infected chickens exhibited anorexia and signs of depression at 2 days postinoculation (DPI). The Q1 inoculated group died within 4 DPI, Q26 within 3 DPI, and Q39 within 2 DPI; contact

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Porcine Astrovirus Type 3 in Central Nervous System of Swine with Polioencephalomyelitis

Technical Appendix

Additional Methods

Tissues were routinely processed at the Iowa State University Veterinary Diagnostic Laboratory for histologic examination. Central nervous system tissues (cerebrum; cerebellum; brainstem; and cervical, thoracic, and lumbar spinal cord) from each animal were independently reviewed by 2 veterinary diagnostic pathologists.

Technical Appendix Table 1. Amino acid differences between PoAstV3/USA/IA/7023/2017 and PoAstV3/USA/US-MO123/2011*

Genomic region, nucleotide position	Porcine astrovirus isolate, aa	
	USA/IA/7023/2017	USA/US-MO123
ORF1ab		
10	K	R
15	T	A
18	T	V
25	L	S
37	D	N
89	D	E
94	D	E
108	Y	W
112	K	N
122	L	S
129	K	R
132	Y	H
148	V	A
160	I	A
170	L	M
177	H	Y
216	D	G
217	G	D
230	F	L
295	F	L
300	F	L
320	I	T
413	R	K
473	T	P
523	A	V
586	R	K
610	S	N
657	H	R
694	L	F
713	T	A
748	A	T
827	K	R
856	H	Y
865	G	E

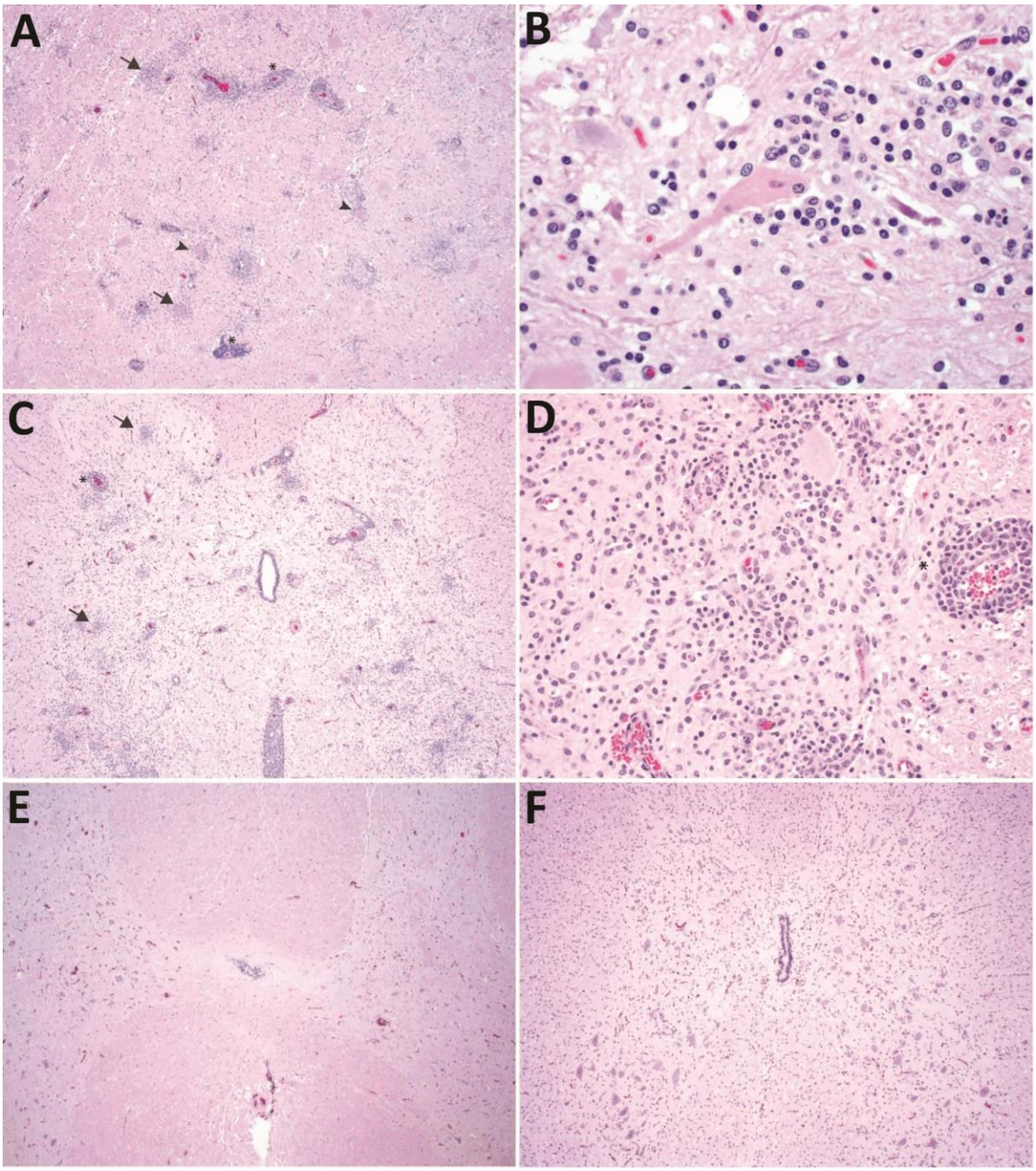
Genomic region, nucleotide position	Porcine astrovirus isolate, aa	
	USA/IA/7023/2017	USA/US-MO123
913	A	T
944	K	R
959	T	A
993	K	R
1024	E	V
1074	D	N
1075	A	D
1110	K	R
1196	C	R
1223	I	T
1334	R	G
ORF2		
17	I	T
23	I	T
24	S	F
25	L	W
44	F	S
57	E	A
68	P	L
107	S	N
114	R	K
120	N	S
197	D	G
251	S	N
333	K	Q
348	I	L
355	A	V
415	A	T
417	E	G
489	H	Y
496	A	V
497	G	N
499	T	N
501	S	T
502	P	H
504	S	P
513	L	K
519	N	D
528	S	P
531	G	P
556	S	A
576	D	N
585	N	A
601	N	D
614	N	E
633	S	N
635	N	D
644	D	N
658	T	S
663	H	S
684	A	T
707	L	P
710	T	A
711	A	V
725	T	N
726	F	Y
738	F	S
752	H	Q
763	N	Y

*ORF, open reading frame.

Technical Appendix Table 2. Porcine astrovirus type 3 quantitative real-time PCR results, by animal and sample type*

Animal ID	Histologic lesions†	Sample type, Cq				
		Cerebrum	Cerebellum	Cervical SC	Thorcic SC	Lumbar SC
Sow A	+	30.33	29.97	24.30	27.28	32.01
Sow B	–	U	U	U	U	U
Sow C	+	30.06	25.76	22.43	27.92	29.63
Sow D	+	U	U	34.92	35.87	38.61
Piglet A	–	U	U	U	U	U
Piglet B	+	30.77	ND	22.58	23.05	27.09

*Cq, quantification cycle value; ID, identification; ND, not done; SC, spinal cord; U, undetectable after 40 cycles.
†Presence (+) or absence (–) of polioencephalomyelitis.



Technical Appendix Figure. Hematoxylin eosin stain of neuronal tissue of swine infected with porcine astrovirus type 3. A) Brainstem from sow C with rhombencephalitis. B) Neuronal necrosis in the brainstem of sow A. C) Nonsuppurative myelitis in piglet B. D) Focal area of gliosis, neuronal satelliosis, and neuronal necrosis in the spinal cord of piglet B. E) Histologically normal spinal cord of sow B. F) Histologically normal spinal cord of piglet A. Asterisk indicates expansion of the Virchow-Robin spaces by lymphocytes and plasma cells. Arrows show multifocal areas of gliosis. Arrow heads show neuronal necrosis.