

Electronic Supplementary Material

Three Novel Avastroviruses Identified in Dead Wild Crows

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Table S1. Primers used for detection of influenza A virus, West Niles virus, and Newcastle disease virus

Primer name	Purpose of pathogens	Sequence (5'→3')	Position	Product size (bp)
FluA-M1-229F	FluA (Bi <i>et al.</i> 2016)	TTCTAACCGAGGTCGAAAC	8–26	
FluA-M1-229R		AAGCGTCTACGCTGCAGTCC	217–236	229
WNV-O-1401-F		ACCAACTACTGTGGAGTC	1401–1418	
WNV-O-1845-R	WNV (Johnson <i>et al.</i> 2001)	TTCCATCTTCACTCTACACT	1466–1485	445
WNV-I-1485-F		GCCTTCATACACACTAAAG	1485–1503	
WNV-I-1732-R		CCAATGCTATCACAGACT	1715–1732	248
Class I NDV_F	NDV- I (Wu <i>et al.</i> 2011)	CACCAAGCTGGAGAAAGGGCATAC	4285–4308	
Class I NDV_R		CAGTATGTTGCAGCATTCTGGTGG	4986–5011	727
Class II NDV_F	NDV- II (Yang <i>et al.</i> 1999; Zhang <i>et al.</i> 2015)	CCATTGCTAAATACAATCCTTCA	4353–4376	
Class II NDV_R		CTGCCACTGCTAGTTGTGATAATCC	5036–5060	708

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Table S2. Primers for whole genome amplification of the novel astroviruses

Strain	Primer	Primer name	Sequence (5'→3')	Product size (bp)
AstV-CAS001	1	CrAstV-CAS001-1F	AGAAGGGATGGCTTTGCAA	
		CrAstV-CAS001-1R	GTATCTCTTGGTAATTGCGT	340
	2	CrAstV-CAS001-2F	GAAGGGATGGCTTTGCAAGT	
	2	CrAstV-CAS001-2R	TCTTCTGCTCGGGCTCCATA	578
		CrAstV-CAS001-3F	TATGTATGGAGCCGAGCAG	
	3	CrAstV-CAS001-3R	TGGTATAGTTGAGTCCTGCTATTG	770
	4	CrAstV-CAS001-4F	TCTCCACTTCATGGTTACGCC	
	4	CrAstV-CAS001-4R	ATTGTTGTTGTCAGTGCTGCAT	768
		CrAstV-CAS001-5F	CAATGGATGAGATGCTGTACAAA	
	5	CrAstV-CAS001-5R	TCGAAACCAGCCTGTCAACT	743
	6	CrAstV-CAS001-6F	CAGTACCGGAGCCCTATCCT	
	6	CrAstV-CAS001-6R	TGGTAGTTCTTGAGTTTGACCG	778
		CrAstV-CAS001-7F	ACCAAGAGAACTTTCCACCA	
	7	CrAstV-CAS001-7R	ACCTCCGGAAGTTCTATGTTCT	702
	8	CrAstV-CAS001-8F	CTTGTGCAACTACGCATCCTC	
	8	CrAstV-CAS001-8R	ACCACCATCCTTGTCTGGGT	750
		CrAstV-CAS001-9F	AGGCGTGGAAAATACCAGCA	
	9	CrAstV-CAS001-9R	TCCACTCATTGGTTCACAAAGG	777
	10	CrAstV-CAS001-10F	GCCAACGGGAATAGGACTT	
	10	CrAstV-CAS001-10R	TGGGGTTGGCTTGATCTCT	779
		CrAstV-CAS001-11F	AACACACAAAGTATGAGGAT	
	11	CrAstV-CAS001-11R	CTTCTGGTGGTGTGGGTTT	336
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Strain	Primer	Primer name	Sequence (5'→3')	Product size (bp)
AstV-CAS002	1	CrAstV-CAS002-1F	CTGGATGGCTTTGAAAGTGTAA	
		CrAstV-CAS002-1R	AGCAACCACTATCCACGCAA	550
	2	CrAstV-CAS002-2F	ACGCAGAAACTATGTGCAGAC	
	2	CrAstV-CAS002-2R	ACACTAGCATTAGCATCCACGA	764
		CrAstV-CAS002-3F	GCAGTTGTTGTCTTTAGCATCTG	
	3	CrAstV-CAS002-3R	GCCTTGAGCAACTACACCTTG	780
	4	CrAstV-CAS002-4F	ACAGGTTGGTTACAGTAGATGG	
	4	CrAstV-CAS002-4R	CGCCTACATTGCTAACATCAT	764
		CrAstV-CAS002-5F	CCTTGATTGTAAGAACTGTGGCA	
	5	CrAstV-CAS002-5R	CTTGCTAAAAGATGCTCCCA	764
	6	CrAstV-CAS002-6F	TGATTATGTGTACCGACCCAGT	
	6	CrAstV-CAS002-6R	ACAGAGCTAACAAATCCGGCA	780
		CrAstV-CAS002-7F	AGTGGTTTATGGGCATTCCA	
	7	CrAstV-CAS002-7R	AGACCATTGACCCAGTGACA	754
	8	CrAstV-CAS002-8F	TCGTGCTAGCCAATACGATCT	774

	CrAstV-CAS002-8R	TAACCTTCTGGTAGTGCTGC		
9	CrAstV-CAS002-9F	CCTGGGGCTGGCTATTGAA	772	
	CrAstV-CAS002-9R	AATTCGGTTGACTGGTGTCTCT		
	CrAstV-CAS002-10F	GGGGTTATCCACACAGCAAGA		
10	CrAstV-CAS002-10R	CCGTGAGCTAGGGAGGTTTA	728	
	CrAstV-CAS002-11F	CTGTACATTCAAGGAAAGGAA		
11	CrAstV-CAS002-11R	GTCAAATTTCCATTATTGATTAG	340	
Strain	Primer	Primer name	Sequence (5'→3')	Product size (bp)
AstV-CAS003	1	CrAstV-CAS003-1F	GATGGCTTTGAAAGTGTGA	338
		CrAstV-CAS003-1R	CATACGCTCTGGCGTAAG	
	2	CrAstV-CAS003-2F	TGAAAGTGTGAATAAGCGTGC	586
		CrAstV-CAS003-2R	GTCACACTGTTCCGCATTGG	
	3	CrAstV-CAS003-3F	CTCTTGGGTGTTGCTGCCA	775
		CrAstV-CAS003-3R	AATTAAACCCAGCCATCGCTATC	
	4	CrAstV-CAS003-4F	AGTTGTGCACTATCTAGCTTTCC	774
		CrAstV-CAS003-4R	TCAATTGTTGAAGTCAAGGCAG	
	5	CrAstV-CAS003-5F	CTGCTTATAAACTCATAGATGGCA	773
		CrAstV-CAS003-5R	TGGGTCATCGACTTGTGTTGTT	
	6	CrAstV-CAS003-6F	AGTTGGCCATATACCAGTTCCA	773
		CrAstV-CAS003-6R	ACCTAGCCAGGTTTCCTTGT	
	7	CrAstV-CAS003-7F	AGCTCTTTCCACATTAAGATGAT	774
		CrAstV-CAS003-7R	TCTCCTCTGTGGTTCCGGT	
	8	CrAstV-CAS003-8F	TGGACGGGAGGACCGAAATA	776
		CrAstV-CAS003-8R	AGGGGCGACTCATTGTTT	
	9	CrAstV-CAS003-9F	ACTGTCAATGTTCTCGGATCAAC	772
		CrAstV-CAS003-9R	AGGAAGGTAATGGCTCATGGT	
	10	CrAstV-CAS003-10F	TGGCTTCCTCTTGAAGGATG	775
		CrAstV-CAS003-10R	ACTTGTGAAAGTGTGTTGTCA	
	11	CrAstV-CAS003-11F	GCAGCGTATTCAAGGCAAGG	659
		CrAstV-CAS003-11R	AGTAGTGATTATCGGGTGATTCC	
	12	CrAstV-CAS003-12F	CAAATGTTGACAACACACTT	341
		CrAstV-CAS003-12R	AGTCAATTCAAGTTTGATT	

Table S3. Specific primers used in this study for detection of the novel astroviruses

RT-PCR/qRT-PCR Primer name	Sequence (5'→3')	Product size (bp)
CrAstV-CAS001F	CTCCGAAGCTAAACCAACTGA	
CrAstV-CAS001R	GCTCCATACATAAATGCCAGAA	303
CrAstV-CAS002F	CAAAGCACTCAGAGCACCCA	
CrAstV-CAS002R	CTTGCCACAGTTCTTACAATCAA	378
CrAstV-CAS003F	TATGATAAGGATAACACCAACCGAAGA	
CrAstV-CAS003R	TGGCAGCAAACACCCAAAGA	315
CrAstV-CAS001qF	AGGAAATAGCACACCTAACAGAAA	
CrAstV-CAS001qR	ATAAATGCCAGAAAGAGGATCA	100
CrAstV-CAS001qP	CAACACGAAAGCACAGTTAGCACG	
CrAstV-CAS002qF	GAATATGCGTATATGCAGGATACA	
CrAstV-CAS002qR	ATGCTGCATATAATCTTCTTCTGT	123
CrAstV-CAS002qP	CGGATAAAAATGTCTTAAGCACACCA	
CrAstV-CAS003qF	CAGGATGGTATGGAGTTAGG	
CrAstV-CAS003qR	TGGCAGTGGTGCTGGTGTAA	104
CrAstV-CAS003qP	TCAATGATCCAAACTTGGCGAA	

Table S4. RT-PCR detection results of astroviruses in different organs of the five dead crows

Crow	Organ	CrAstV-CAS001	CrAstV-CAS002	CrAstV-CAS003
Crow 1	Heart	-	-	-
	Liver	-	-	+
	Spleen	-	-	+
	Lung	-	-	+
	Kidney	-	-	-
	Intestine	-	-	+
Crow 2	Brain	-	-	-
	Heart	-	-	-
	Liver	-	-	-
	Spleen	+	+	-
	Lung	-	-	-
	Kidney	+	-	-
Crow 3	Intestine	+	+	-
	Brain	-	-	-
	Heart	-	-	-
	Liver	-	-	-
	Spleen	-	-	-
	Lung	-	-	-
Crow 4	Kidney	-	-	-
	Intestine	-	-	-
	Brain	-	-	-
	Heart	-	-	-
	Liver	-	-	-
	Spleen	-	-	-
Crow 5	Lung	-	-	-
	Kidney	-	-	-
	Intestine	-	-	-
	Brain	-	-	-
	Heart	-	-	-
	Liver	-	-	-
Crow 5	Spleen	-	-	-

+, RT-PCR positive; -, RT-PCR negative.

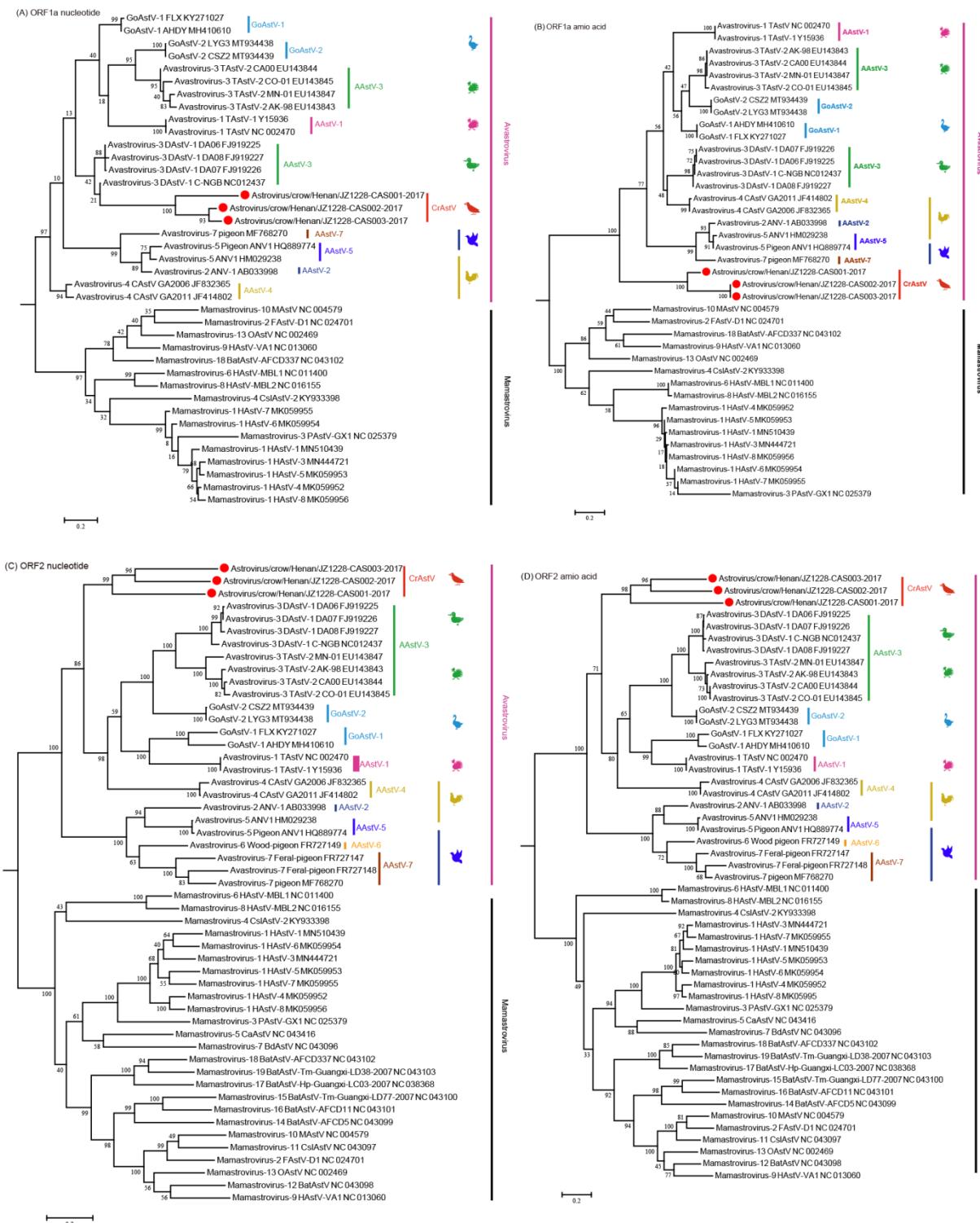


Fig. S1 Phylogenetic analysis of astroviruses. **A** Nucleotide sequence of *ORF1a* gene. **B** Amino acid sequence of *ORF1a* gene. **C** Nucleotide sequence of *ORF2* gene. **D** Amino acid sequence of *ORF2* gene. The red points highlighted in the phylogenetic tree represent the viruses identified in the present study. The labels on the right represent different genotypes of *Astrovirus*.

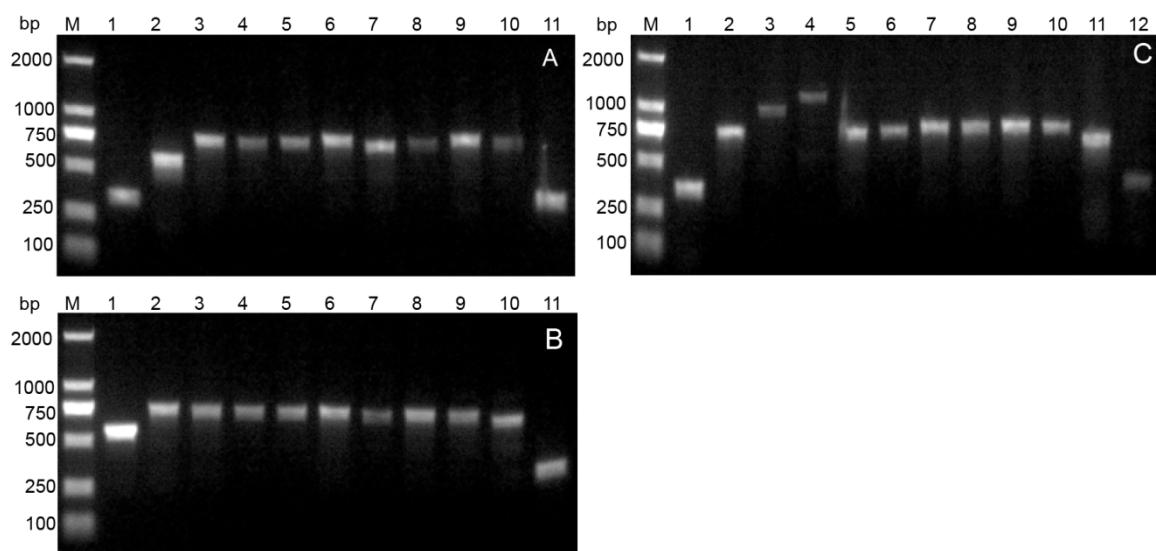


Fig. S2 Amplification of the three novel astroviruses by RT-PCR. **A** Amplification of AstV-CAS001 from the spleen of crow 2, **B** Amplification of AstV-CAS002 from the spleen of crow 2, **C** Amplification of AstV-CAS003 from the intestine of crow 1.