Table S1: Details on oligonucleotide sequences, orientation and binding site to the BoAstV-CH13 genome.

Oligonucleotide	Sequence	Orientation	Binding site	Application
BAstV_qR	5'-ACA ACC TCC TTG GCA ATC TG-3'	-	4223-4204	Reverse transcription
BAstV_qL_PstI	5'-GCT AGT CTG CAG TTT TGG CTC GTC ACT TTG TG-3'	+	4059-4076	Cloning ISH Probe A
BAstV_qR_EcoRI	5'-AAG CTC GAA TTC ACA ACC TCC TTG GCA ATC TG-3'	-	4223-4204	Cloning ISH Probe A
BAV_3_PstI	5'-GCT AGT CTG CAG ACC GCC TTT CCG ATG ATG TAC-5'	+	4951-4972	Cloning ISH Probe B
BAV_4_EcoRI	5'-AAG CTC GAA TTC ATC AAC AAC CTG CCA TAT-3`	-	5123-5103	Cloning ISH Probe B
BAV-3	5'-ACC GCC TTT CCG ATG ATG TGC	+	4952-4972	Cloning for sequencing
BAstV_Orf2_R_new	5'-CTC GGC GTG GCC TCG G-3`	-	6438-6423	Cloning for sequencing
MA 4	5'-TGG ACC CGC TAT GAT GGC ACI AT-3`	+	3378-3400	Cloning for Sequencing, RT-PCR
MA 2	5'-GGC TTT ACC CAC ATI CCA AA-3'	-	3805-3786	RT-PCR
BAV_4	5'-TTC ATC AAC AAC CTG CCA TAT-3`	-	5124-5104	Cloning for sequencing
BAV 9-4 fo	5'-TGA CAT TGA TGC TGA GTG G-3`	+	2229-2247	Cloning for sequencing
BAV 10-4 do	5'-CCG ATG TTG TTC AGT AAC TTC-3`	-	3560-3540	Cloning for sequencing
BAV 11-5 fo	5'-CTG GTC TGT TTC TTA ATG ATG C-3`	+	1046-1067	Cloning for sequencing
BAV 12-5 do	5'-GGT ATT GAC AAG TTC CTC AGA-3`	-	2313-2293	Cloning for sequencing
BAV 13-6 fo	5'-GTC CAA GAA TGG CTC TTT CA-3`	+	44-63	Cloning for sequencing
BAV 14-6 do	5'-TGT GCT TTC ACC ATT ATA GCA-3`	-	1094-1074	Cloning for sequencing
BAstV_Orf2_R	5'-ATG ATC ACT GAA GTA AAC AAA TCC-3'	-	6481-6456	Reverse transcription
Gene Racer Oligo d(T)	5′-GCTGTCAACGATACGCTACGTAACGGCATGACAGTG$(T)_{24}$-3′	-	PolyA-tail	3'RACE
Gene Racer 3`	5'-GCT GTC AAC GAT ACG CTA CGT AAC G-3′	-	-	3'RACE
BAV_5	5'- ATG AGG GCG TGC GTG GTG TTC-3'	+	6359-6379	3'RACE
5'RACE AAP	5'- GGC CAC GCG TCG ACT AGT ACG GGI IGG GII GGG IIG-3'	+	-	5'RACE
BAV 20	5'- ACT CGT TCC CAG CAA CTA CAC	-	313-293	5'RACE
5'RACE AUAP	5'- GGC CAC GCG TCG ACT AGT AC-3'	+	-	5'RACE nested PCR
BAV 16	5'- ATC GCC GGT GGG GTC AAT CA-3'	-	252-233	5'RACE nested PCR

Table S2: Results of the bioinformatics pipeline for viral protein identification applied to NGS data of three cows with non-suppurative encephalitis of unknown etiology.

Case ID	Library	Contigs		BLASTx hits				Alignment	
		Total	Mapped/length	Virus family	Virus	Protein*	GeneBank Accession Number	Similarity [%]	Length [aa]
42145	DNA	24542	1/992	*Retroviridae*	Human betaretrovirus	Pol	AFZ15778.1	52.94	255
					Mouse mammary tumor virus C	Pol	P11283.2	53.36	253
					Mouse mammary tumor virus B	Pol	P03365.2	52.94	255
	RNA	7625	-	-	-	-	-	-	-
45641	DNA	36176	1/1739	*Retroviridae*	Human betaretrovirus	Pol	AFZ15778.1	51.76	255
					Mouse mammary tumor virus C	Pol	P11283.2	52.17	253
					Mouse mammary tumor virus B	Pol	P03365.2	51.76	255
	RNA	4373	-	-	-	-	-	-	-
45664	DNA	21367	-	-	-	-	-	-	-
	RNA	1922	1/6540	*Astroviridae*	Astrovirus VA3	RdRp	AFV53437.1	63.31	556
					Astrovirus_VA3	Capsid	YP_006905860.1	59.63	436
					Astrovirus VA3/HMO	RdRp	AGF91827.1	65.45	275
					Astrovirus_VA3/HMO	Capsid	AGF91828.1	60.49	205
					Astrovirus VA4	RdRp	AFV53440.1	65.34	528
					Fox astrovirus	RdRP	AGK45543.1	57.65	510
					Fox astrovirus	Capsid	AGK45544.1	63.11	412
					Human astrovirus 2	RdRp	AFR23485.1	50.2	253
					Human astrovirus 5	NS1ab	Q4TWH8.2	50.1	525
					Mink astrovirus 1	NS1ab	Q80KJ7.2	59.62	520
					Mink astrovirus 1	Capsid	Q80KJ6.1	63.99	386
					Ovine astrovirus 1	Capsid	Q9JH65.1	66.58	769
					Ovine astrovirus 1	NS1a	Q9JH67.1	55.92	853
					Ovine astrovirus 1	NS1ab	Q9JH66.2	69.46	854
					Porcine astrovirus_2	NS1ab	ADO30543.1	50.1	523
					Porcine astrovirus_3	NS1ab	AFW16976.1	61.93	528

*Pol, retrovirus polyprotein; RdRp, RNA dependent RNA polymerase; NS, non-structural proteins

Materials and Methods

In situ hybridization (ISH). BoAstV_CH13 cDNA was amplified by PCR with primers BAstV_qL_PstI and BAstV_qR_EcoRI for Probe A, and with primers BAV3_PstI and BAV4_EcoRI for Probe B (see Table S1 in the supplemental material). The resulting amplicons were cloned into the pSPT_18 plasmid (Roche) via the PstI and EcoRI restriction sides. Inserted sequences were confirmed by Sanger sequencing, plasmids were linearized with PstI (Probe A) or HindIII (Probe B), and DIG-labeled antisense RNA was transcribed with T7 RNA polymerase. Tissue slides of BoAstV-CH13 positive and negative brain tissues were deparaffinized, rehydrated, incubated in 0.2 M HCl for 20 min, and treated with Proteinase K (5 µg/ml [Roche] in 0.05 M Tris-HCl, 0.15 mM CaCl2) to increase target accessibility. Proteinase K was inactivated by 4% (w/v) paraformaldehyde, and the tissues were acetylated with 0.25% (v/v) acetic anhydride. Next, the slides were pre-incubated in hybridization mix (50% [v/v] deionized formamide, 0.05% [w/v] Ficoll, 0.05% [w/v] polyvinylpyrrolidone, 0.05% [w/v] bovine serum albumin [BSA], and 4 × saline-sodium citrate), supplemented with 0.25% (w/v) yeast RNA, for 2 h at 50°C. Probe hybridization was accomplished in hybridization mix supplemented with 0.5% (w/v) yeast RNA, 10% (w/v) dextran sulfate, and ~1 ng/ml of DIG-labeled RNA (Probe A, Probe B, or an unrelated control probe) for ~16 h at 50°C. Excess labeled RNA was removed by intensive washing and RNase treatment for 30 min at 37°C. Finally, binding of DIG-labeled RNA probe was detected with anti-digoxigenin alkaline phosphate conjugated Fab fragments (Roche) and a nitro-blue tetrazolium / 5-bromo-4-chloro-3’-indolyphosphateubstrate (Roche).