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SHORT COMMUNICATION

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Astrovirus VA1 in patients with acute gastroenteritis

Gianvito Lanave[1] | Daniela Loconsole[2] | Francesca Centrone[2] | Cristiana Catella[1] | Paolo Capozza[1] | Georgia Diakoudi[1] | Antonio Parisi[3] | Elisabetta Suffredini[4] | Alessio Buonavoglia[5] | Michele Camero[1] | Maria Chironna[2] | Vito Martella[1]

[1]Department of Veterinary Medicine, University of Bari, Valenzano (Bari), Italy

[2]Department of Biomedical Sciences and Human Oncology-Hygiene Section, University of Bari, Bari, Italy

[3]Istituto Zooprofilattico Sperimentale di Puglia e Basilicata, Sezione di Putignano, Bari, Italy

[4]Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy

[5]Department of Biomedical Sciences and Human Oncology, Unit of Internal Medicine 'Guido Baccelli', University of Bari, Bari, Italy

Correspondence
Vito Martella, Department of Veterinary Medicine, University of Bari, Strada Provinciale per Casamassima km3, 70010, Valenzano (Bari), Italy.
Email: vito.martella@uniba.it

Abstract

Human astroviruses (AstVs) are usually associated with acute gastroenteritis. In recent years, atypical animal-like AstVs have been identified, but their pathogenic role in humans has not been determined. Starting from 2010, there has been a growing evidence that AstVs may also be associated with encephalitis in human and animal hosts. Some human atypical AstV strains (VA1, MLB1/MLB2) display neurotropic potential, as they have been repeatedly identified in patients with AstV-related encephalitis, chiefly in immunosuppressed individuals. In this study, a VA1-like AstV was identified from a single stool sample from an outbreak of foodborne acute gastroenteritis occurred in Italy in 2018. On genome sequencing, the virus was related to the VA1-like strain UK1 (99.3% at the nucleotide level). Similar viruses were also found to circulate in paediatric patients hospitalized with AGE in the same time span, 2018, but at low prevalence (0.75%, 3/401). Gathering epidemiological data on atypical AstVs will be useful to assess the risks posed by atypical AstV infections, chiefly in medically fragile patients.

KEYWORDS

astrovirus, foodborne, gastroenteritis, human, neurotropism, VA1

1 | INTRODUCTION

Human astroviruses (HAstVs), family *Astroviridae*, are causative agents of acute gastroenteritis (AGE) with worldwide distribution (Mendez and Arias, 2013). Although HAstVs are a common cause of viral gastroenteritis in paediatric population, they have been also identified in adult and elderly and they have also been associated with large foodborne outbreaks (Mendez and Arias, 2013). In immunocompromised hosts, they can cause chronic infections and extra-intestinal disease with severe complications (Cortez et al., 2017; Reuter et al., 2018).

Human infections are predominantly caused by the mamastrovirus species 1 (MAstV-1), also referred to as 'classic' HAstVs (Mendez and Arias, 2013). In the last decade, genetically divergent HAstV species, referred to as 'atypical' or 'animal-like' strains, of probable zoonotic origin, have been discovered, including strain Melbourne (MLB) (species MAstV-6) (Finkbeiner, Allred, et al., 2008, Finkbeiner, Kirkwood, et al., 2008), strain Virginia/Human-Mink-Ovine-like (VA/HMO) (species MAstV-8 and MAstV-9) (Jiang et al., 2013; Kapoor et al., 2009; Finkbeiner et al., 2009) and the tentative species MAstV-20 (Meyer et al., 2015) (Table 1). Thus far, a firm association between these novel HAstVs and AGE has not been established (Vu et al., 2017), although epidemiologic studies have confirmed the presence of atypical HAstVs worldwide (Reuter et al., 2018). In addition, both classical and atypical HAstVs have been associated with either respiratory illness (Cordey et al., 2016) or central nervous system (CNS) infections in vulnerable subjects (Reuter et al., 2018; Vu et al., 2017; Koukou et al., 2019; Schibler et al., 2019).

In April 2018, we monitored an AGE outbreak related to the consumption of raw seafood in Bari, Apulia Region, Italy. The outbreak affected a group of 30 persons, and two-thirds presented with AGE signs. Fifteen samples from symptomatic and asymptomatic individuals involved in the outbreak were collected and screened for viral enteric pathogens revealing multiple viral

TABLE 1 Naming and nomenclature scheme of human astroviruses. Astroviruses associated with central nervous system (CNS) disease are indicated (modified from Vu et al., 2017). In brackets, the number of cases of CNS infection reported as of September 2020

Common name	Species	Geno/ serotype	CNS disease
Classic	MAstV-1	HAstV-1	(1)
		HAstV-2	-
		HAstV-3	-
		HAstV-4	(1)
		HAstV-5	-
		HAstV-6	-
		HAstV-7	-
		HAstV-8	-
Animal-like or atypical	MAstV-6	MLB1	(1)
		MLB2	(3)
		MLB3	-
	MAstV-8	VA2(HMO-A)	-
		VA4	-
	MAstV-9	VA1(HMO-C)	(5)
		VA3 (HMO-B)	-
	MAstV-20*	VA5	-

*Candidate species.

infections including norovirus, kobuvirus and classical and atypical (VA1-like) AstVs. In order to evaluate the presence of VA1 AstV in local population in the same time span, we also screened a total of 401 faecal samples from young patients hospitalized with AGE in Bari during 2018.

2 | MATERIALS AND METHODS

In April 2018, an AGE outbreak related to the consumption of raw seafood in a restaurant in Bari, Apulia Region, Italy, was reported. The outbreak affected a group of 30 persons, 20 (66.6%) of which presented with AGE signs. A total of 6 stools samples from symptomatic and 9 from asymptomatic subjects were collected and screened for viral enteric pathogens (rotavirus, adenovirus, norovirus, astrovirus and kobuvirus).

AstV infection was initially identified using a quantitative molecular assay specific for MAstV-1 in 5/6 patients with AGE and 2/9 asymptomatic subjects (Table 2). All the 15 collected specimens were re-tested using a broadly reactive (pan-astrovirus) nested RT-PCR protocol able to detect different species of AstVs, designed on the RdRp of AstVs (Chu et al., 2008).

The complete genome of strain ITA/2018/205.18-5 was reconstructed using 5′ and 3′ rapid amplification of cDNA ends (RACE) protocols and a 'primer walking' strategy (Brown et al., 2015).

TABLE 2 Results of the screening for AstV in patients from the foodborne outbreak of acute gastroenteritis (AGE)

Patient		RT-PCRs for AstV		ORF1b/ORF2 sequencing		RT-qPCRs	
Age (years)	AGE signs	ORF1b[a]	ORF2[b]	Species	Type	VA1[c] (Ct)	MAstV-1[d] (Ct)
30	+	+	+	MAstV-1	HAstV-1	-	+ (16)
35	+	+	+	MAstV-1	HAstV-1	-	+ (18)
34	+	+	+	MAstV-1	HAstV-1	-	-
25	+	+	+	MAstV-1	HAstV-1	-	+ (17)
7	+	+	-	MAstV-9	VA1	+ (20)	+ (36)
34	+	+	-	MAstV-1	HAstV-3	-	+ (26)
35	-	+	-	MAstV-1	HAstV-1	-	+ (36)
35	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-
35	-	+	+	MAstV-1	HAstV-1	-	+ (36)
64	-	+	+	MAstV-1	HAstV-1	-	-
35	-	+	+	MAstV-1	HAstV-1	-	-
63	-	-	-	-	-	-	-
24	-	+	-	MAstV-1	HAstV-1	-	-
35	-	-	-	-	-	-	-

Abbreviations: MAstV-1, mamastrovirus species 1; RT-qPCR, quantitative reverse transcription RT-PCR, Ct cycle threshold.

[a]Pan-astrovirus hemi-nested RT-PCR targeted to ORF1b region (RdRp).

[b]RT-PCR targeted to ORF2 region (capsid) of MAstV-1.

[c]Quantitative RT-PCR specific for VA1 AstV (MAstV-9).

[d]Quantitative RT-PCR specific for classic human AstVs (MAstV-1).

Since mixed/multiple viral infections were identified in stool samples from the foodborne AGE outbreak, the samples were re-screened using a highly specific RT-qPCR designed on ORF1b genomic region of VA1 (Brown et al., 2015).

Additionally, in order to evaluate the presence of VA1 AstV in local population in the same time span (January–December 2018), 401 faecal samples from young patients aged between 1 month and 21 years were screened with the VA1-specific RT-qPCR assay. The samples were collected from patients hospitalized with AGE at the Pediatric Hospital of Bari.

The research was conducted in accordance with the ethical standards of the institutional and national research committees, under the principles of the 1964 Helsinki Declaration and its later amendments. The samples collected in the study are part of a public health surveillance, instituted by Regional Decree of Apulia Region nr 565 of April 2, 2014, for gastroenteritis in Apulia Region. Subjects and each parent or legal guardian of children who provided samples signed a written informed consent.

The Bayesian phylogenetic analysis on partial ORF-1b sequences of AstVs retrieved from this study and a selection of cognate sequences retrieved from the GenBank database was carried out with Geneious v. 9.1.8 (Biomatters, New Zealand).

3 | RESULTS AND DISCUSSION

On screening with multiple primer sets for AstVs, the samples collected from the outbreak displayed different patterns of reactivity (Table 2), as a combined result of different virus load among the samples, different sensitivity and specificity of the various assays and of mixed infections. Overall, AstV RNA was detected in 11/15 samples of the foodborne AGE outbreak. On sequencing of the RNA-dependent RNA polymerase (RdRp) and/or ORF2 regions, 10 samples were characterized as MAstV-1, type 1 (nr = 9) and type 3 (nr = 1), whilst a unique sample was characterized as MAstV-9 (Table 2).

Sequence analysis of the MAstV-9 strain (ITA/2018/205.18-5) revealed the highest nucleotide (nt) identity (99.3%) to the VA1 AstV strain UK1 (GenBank Accession nr KM358468). The samples from the AGE outbreak also tested positive for norovirus and for aichivirus (Table S1), mostly in co-infections, suggesting heavy contamination of the food source.

The genome of strain ITA/2018/205.18-5 (GenBank Accession nr MT432184) was generated by deep sequencing with a mean coverage of 310X, and reference assembling using to the prototype VA1 strain UK1. The genome sequence was 6586 nt long and included an untranslated portion (UTR) of 38 nt at the 5' end, three open reading frames (ORFs), 1a, 1ab and 2, with a length of 2661 nt, 4185 nt and 2277 nt, respectively, and a 98-nt long UTR region at the 3' end. Nucleotide identity to strain UK1 across the 3 ORFs ranged between 99.1 and 99.3%. Overall, there was a limited genetic variability with other VA1-like genome sequences (93.8-99.3% nt identity).

Screening of stool samples from the foodborne AGE outbreak by quantitative reverse transcription PCR (RT-qPCR) system confirmed the presence of VA1 virus (cycle threshold Ct = 20) only in a 7-year-old child with AGE signs. The child was also co-infected with an MAstV-1 (Table 2), although this was only evidenced in RT-qPCR (Ct = 36), and with norovirus and aichivirus (Table S1).

Out of 401 faecal samples from young patients of local population in 2018, VA1 AstV was identified in an additional 3 faecal samples (0.75%) collected from patients with age ranging from 10 to 29 months. The Ct values of the 3 VA1-positive samples ranged from 28 to 33, and all the samples tested negative for other enteric viruses and bacterial pathogens. On sequencing of the ORF1b fragment, two strains (ITA/2018/98.20-36 and ITA/2018/98.20-96) displayed the highest nt identity (100%) to strain UK1 (KM358468), whilst one strain (ITA/2018/98.20-391) showed the highest nt identity (98.2%) to strain 15-G0180_Ger (KY250100).

The Bayesian phylogenetic tree revealed the presence of two different clades. Strains ITA/2018/98.20-36 (MT951189) and ITA/2018/98.20-96 (MT951190), detected in early 2018 (January and March), clustered with the AGE outbreak strain ITA/2018/205.18-5, whilst strain ITA/2018/98.20-391 (MT951191), detected in November, clustered with German VA1-like AstVs (Figure 1).

Overall, in our survey the prevalence rate in paediatric population was low (0.75%) and this was in line with the existing literature. Despite the detection of VA1 with direct diagnostic methods is sporadic (≤2.1%) (Vu et al., 2017), serological studies have shown the presence of VA1-specific antibodies in a large portion (65%) of the population (Burbelo et al., 2011). Our data suggest that the VA1 strain detected in the foodborne AGE outbreak was also circulating in local population in the winter–spring season, although its detection in the outbreak appeared serendipitous, as the virus was not detected in other patients from the same foodborne outbreak.

VA1 strain was first discovered in 2009 in stools of patients with AGE in Nepal and the United States (Kapoor et al., 2009; Finkbeiner et al., 2009). In 2010, VA1 strain was identified in the brain tissues of an immunodepressed 15-year-old boy (Quan et al., 2010). Since then, CNS localization of AstVs has been documented in several animal species (Reuter et al., 2018). In spite of the massive genetic heterogeneity of MAstVs, some strains clearly show a neurotropic attitude, and they are repeatedly identified in the CNS of humans and animals. VA1-like viruses account for 45.5% (5/11) of cases of AstV CNS infections reported in humans in the literature, whilst strain MLB1/MLB2 account for 36.4% (4/11) (Vu et al., 2017; Koukou et al., 2019; Schibler et al., 2019). Likewise, strain Neuro S1/CH13 accounts for the majority of cases of MAstV-related sporadic bovine meningoencephalitis (Selimovic-Hamza et al., 2016). Also, poli-encephalomyelitis in pigs seems associated with porcine AstV type 3 (Arruda et al., 2017). Interestingly, experiments in vitro have demonstrated that VA1 is able to grow efficiently in vitro in primary human astrocytes and in neuroblastoma-derived cell SK-N-SH (Janowski et al., 2019).

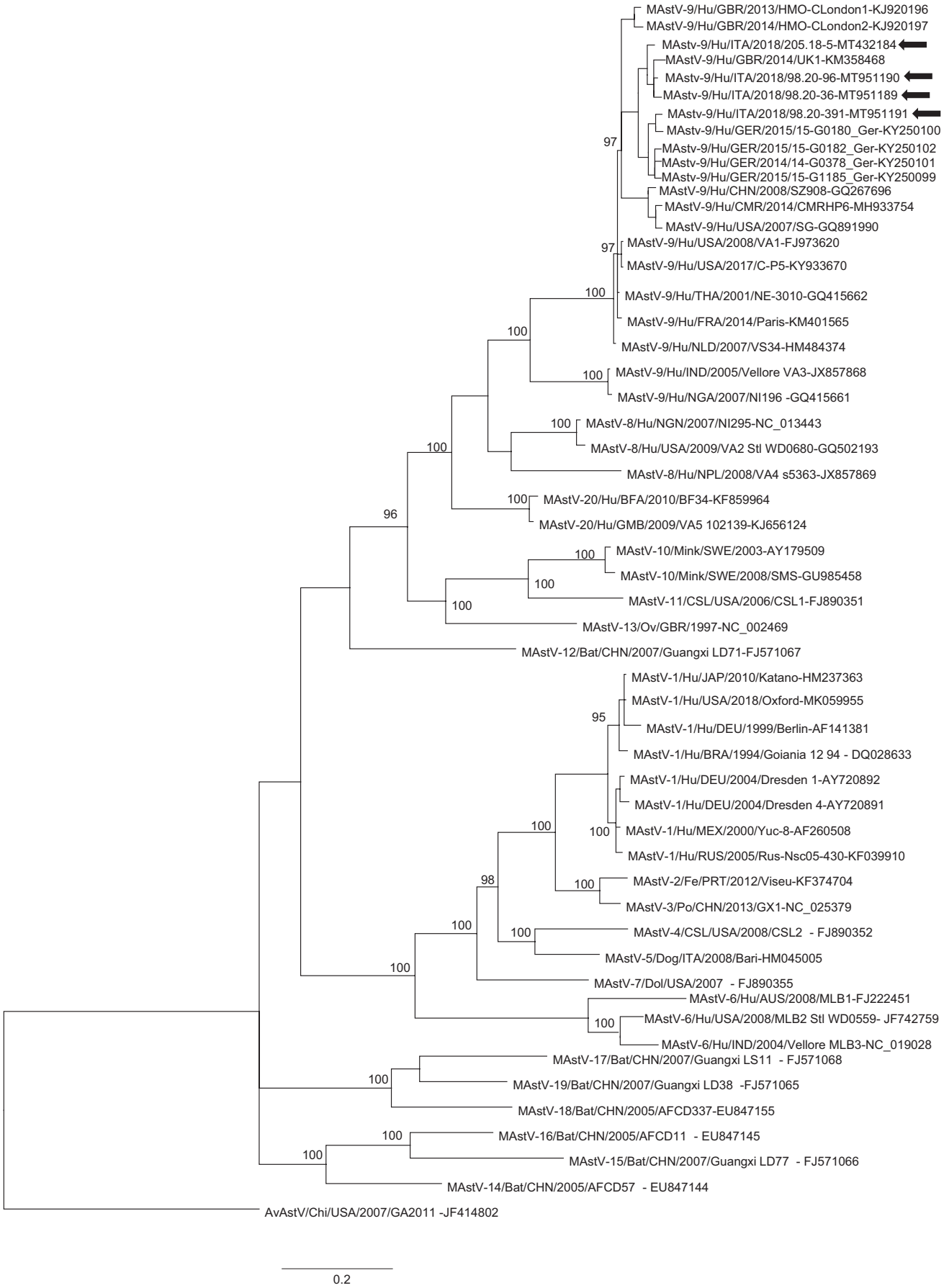

FIGURE 1 Bayesian ORF1b-based phylogenetic tree of AstVs. The tree was constructed using a partial (389 nt) portion of ORF1b. Posterior probability values >95% are indicated at the tree nodes. Black arrows indicate the human AstV strains detected in this study. The scale bar indicates the number of nt substitutions per site. Abbreviations: MAstV, mammalian astrovirus; AvAstV, avian astrovirus

Our study, although unveiling the circulation of atypical AstVs in local population, has several limitations. For instance, the study did not include a control cohort. Also, the study was based on a relatively small geographical basin, during a single year, and covered only the paediatric population. Moreover, the presence of different viral strains in the outbreak in this case could be assessed more effectively using a deep sequencing approach. Gathering epidemiological data on atypical AstVs in larger, structured surveillance studies will be useful to understand how and to which extent they circulate in human population and to assess more precisely the risks posed by atypical AstV infection in hospital environments (Cortez et al., 2017; Cordey et al., 2017) chiefly in wards with immunosuppressed patients. Also, as the majority of human cases of meningitis and encephalitis remain undiagnosed, this information could be useful to target the diagnostics and therapy of AstV-related CNS infections.

CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

All data generated or analysed in this study are included in this published article.

ORCID

Antonio Parisi https://orcid.org/0000-0003-2744-1140
Vito Martella https://orcid.org/0000-0002-5740-6947

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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