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The First Detection and Phylogenetic Analysis of *Bovine Astrovirus* from Diarrheic Calves in Turkey

Turhan Turan, Hakan Işıdan

Sivas Cumhuriyet University, Faculty of Veterinary Medicine, Department of Virology, 58140, Sivas, TURKEY

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Abstract: A total of 127 stool samples (rectal swab) from diarrheic calves, up to one mouth of age, were collected during one year period (2014-16) from three cities (Sivas, Malatya and Elazığ) located central Turkey. 432 bp partial sequence of Nsp1ab gene were amplified. The PCR amplicons were purified and sequenced and deposited to GenBank. Sequence alignment and phylogenetic analysis being based on partial nucleotide sequences of Nsp1ab gene was constructed. As a result of RT-PCR study, we found that the 3.15% of fecal samples (4/127) were positive for diarrhea calves from Turkey. The neighbor-joinning tree of partial sequence of Nsp1ab gene (ORF1a) indicated that strains were substituted under three distinct lineage. None of our strains belonged to the Neuro1 lineage (lineage 3) of *Bovine astrovirus* which was isolated from bovine with neurological symptoms. On the other hand, while BAstVHT1-TUR strain was substituted under lineage 1a, the other novel starts were lineage 2. While the identity of analyzed sequences varies from 51.1 to 100%, novel strains were calculated between 75.8 to 100 % each other. As a result, we report the first detection and phylogenetic analysis of *Bovine astrovirus* from Turkey.

Key words: *Bovine astrovirus*, calf, phylogenetic analysis, Turkey.

Türkiye'de İshalli Buzağılardan *Bovine Astrovirus'un* İlk Teşhisi ve Filogenetik Analizi

Özet: Orta Anadolu'da yer alan üç ilden (Sivas, Malatya ve Elazığ), 2014-16 yılları arasında toplam 127 dışkı örneği toplandı. *Bovine astrovirus'un* Nsp1ab geninin 432 bp kısmi bölgesi çoğaltıldı, dizi analizi yapıldı ve GenBank'a yüklendi. Sekans hizalaması ve filogenetik analizler yapılarak moleküler karakterizasyonu gerçekleştirildi. RT-PCR çalışmasının sonucuna göre örneklerin %3,15'i (4/127) pozitif olarak tespit edildi. Kısmi Nsp1ab gen (ORF1a) bölgesinin neighbour-joinning yöntemiyle filogeni ağacı oluşturuldu. Buna göre izolatlarımızın hiçbiri sinirsel belirti gösteren sığırlardan izole edilen Neuro1 hattında (hat 3) yer almazken BAstV-HT1-TUR suşumuz hat 1a altında ve diğer suşlarımız ise hat 2 altında konumlandı. Analiz edilen sekansların benzerliği %51,1'den %100'e varan oranlarda gerçekleşirken yeni suşların kendi arasındaki benzerlik oranının %75,8'den %100'e vardığı ortaya konuldu. Sonuç olarak, *Bovine astrovirusun* Türkiye'de ilk teşhisi ve filogenetik analizi rapor edilmiştir.

Anahtar sözcükler: *Bovine astrovirus*, buzağı, filogenetik analiz, Türkiye.

Introduction

Neonatal diarrhea of calves is the one of the significant health problem for the cattle industry. A numerous ethnology including as bacterial and viral agents (such as *Bovine rotavirus*; BRV, *Bovine coronavirus*; BCoV, *Bovine viral diarrhea virus*; BVDV, etc.) have been shown that responsible for the diarrhea of calves [3, 8]. Additionally newly emerging viruses, such as *Bovine torovirus* (BToV), *Bovine norovirus* (BoNoV) and *Bovine nebovirus* (BoNeV); also bovine picornaviruses: bovine enterovirus, Aichivirus B and hunnivirus A) were also reported as an enteric agent isolated from calves [3, 8, 17, 18].

The family *Astroviridae* consists of small (28–30 nm), non-enveloped, single-stranded positive-sense RNA viruses of approximately 7 kb in length. They have been classified into two genera, namely Mamastroviruses (MAstVs) and Avastroviruses (AAstVs), are known to infect mammalian and avian species, respectively [4]. Mamastroviruses have a broad host range including humans, mink, sheep, pigs, rats, marine mammals, dogs, cheetahs, roe deer, cattle and bats [2, 9, 10, 13, 19, 23, 25].

Astroviruses are generally associated with either mild or severe enteric disease symptoms such as diarrhea and vomiting in a number of mammalian species [12]. The first reports in animals were from lambs and calves suffering from diarrhea [24, 27].

Yazışma adresi / Correspondence: *Hakan Işıdan, Sivas Cumhuriyet University, Faculty of Veterinary Medicine, Department of Virology, Sivas, Turkey e-mail: hisidan@cumhuriyet.edu.tr*

Subsequently, a bovine enteric virus antigenically related to the UK strain of BoAstV was isolated in Florida from a calf with diarrhea [28]. *Bovine astrovirus* was considered to be avirulent, as experimentally infected gnotobiotic calves remained clinically normal, although pathological studies on infected calves were not performed. Although calves experimentally infected with this astrovirus did not develop clinical disease, the virus caused cytopathology of the M cells of the dome epithelium covering the Peyer's patches [28].

It was originally concluded that in natural conditions, BoAstV does not seem to be directly associated with a severe diarrheic disease in calves [5, 25, 27, 28] and few controversial data are available on the prevalence of this infection. An astrovirus was isolated from diarrheic yaks from Tibet and also a diarrheic European roe deer (Capreolus capreolus), both are genetically related to BoAstV, suggesting that this virus could cross the species barrier to infect both cattle and roe deer or yaks [7, 25]. On the other hand, although astroviruses were mainly found in relation to gastroenteritis in animals and humans so far, recently detected astrovirus infections were also related to encephalitis. [11, 15, 20, 21].

The existence of *Bovine astrovirus* is not yet reported from Turkey. The aim of the study is to observe bovine astroviruses from fecal samples of diarrheic calves in Turkey. In addition that, sequence and phylogenetic analysis of novel strains is also aimed.

Materials and Methods

Samples and RNA isolation: A total of 127 stool samples (rectal swab) from diarrheic calves, up to one mouth of age, were collected during one year period (2014-16) from three cities (Sivas, Malatya, and Elazığ) located central Turkey. During the sampling period collected stool samples transported to the laboratory quickly as possible and stored in minus 80ºC deep freeze until they are submitted to the RNA isolation.

Fecal samples were 1/10 diluted in 1M phosphate buffered saline and centrifuged 5 minutes at 5000 rpm to remove large cellular debris. After the centrifugation, supernatants were submitted to the nucleic acid extraction procedure according to the manufacturer's informations of GF-1 Viral Nucleic Acid Extraction Kit (Vivantis Technologies, Malaysia). Eluted nucleic acids were stored in -80ºC deep-freeze until use.

Reverse transcription polymerase chain reaction (RT-PCR): The cDNA synthesis was carried out in a 25µl final volume containing 4µl of RNA extract, 10mM deoxynucleoside triphosphate (dNTP), 2,5µl 10x RT buffer (50mM Tris-HCl (pH 8.3 at 25°C), 75mM KCl, 3mM MgCl2 and 10mM DTT), 50ng of the random hexamer, 40 U RNasin, 200 U M-MuLV Reverse-Transcriptase RNase H (Vivantis, Germany). The reverse transcription was performed at 37°C for 1 h. Obtained cDNA samples were amplified with redesigned primer set of DPF/DPR which was designed by Tse et al. [25] previously.

Table 1. Primers used in the study.

Primer Name	Sequences (5'-3')	References
DPF	GAYTGGACBCGHTWTGATGG	Tse et al. [25]
DPFr	GAYTGGAC**HM**G**N**TW**Y**GA**Y**GG*	This study
DPR	KYTTRACCCACATNCCAA	Tse et al. [25]

*Redesigned nucleotides indicated as bold lettering.

The PCR was conducted in a 50µl final volume using 5µl of the RT reaction mixture as a template. The PCR mixture contained 5µl 10x PCR buffer, 10 mM dNTP, 10 pmol/µl of each sense/antisense primer, and 5 U of Taq DNA polymerase (Vivantis, Germany). The PCR was conducted under the following conditions: 1 cycle at 95°C for 2 min and 40 cycles of 94°C for 40 s, (primer melting temperature -5°C) for 30 s, and 72°C for 40 s, followed by a final elongation step of 72°C for 10 min. PCR products were analyzed by electrophoresis in 2% agarose gels stained with ethidium bromide.

Sequencing and phylogenetic analysis: The PCR amplicons were purified with a Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI) and sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on an automated sequencer (ABI 3100; Applied Biosystems, Foster City, CA). All of the sequenced products were used to obtain phylogenetic data. Partial sequences of nonstructural protein gene

were compared with other *Bovine astrovirus* sequence datas which are online provided by National Center for Biotechnology Information (NCBI). Sequence alignment and phylogenetic analysis based on partial nucleotide sequences of 423 bp Nsp1ab gene was constructed with the use of a software, Unipro UGENE, version 1.21 [16].

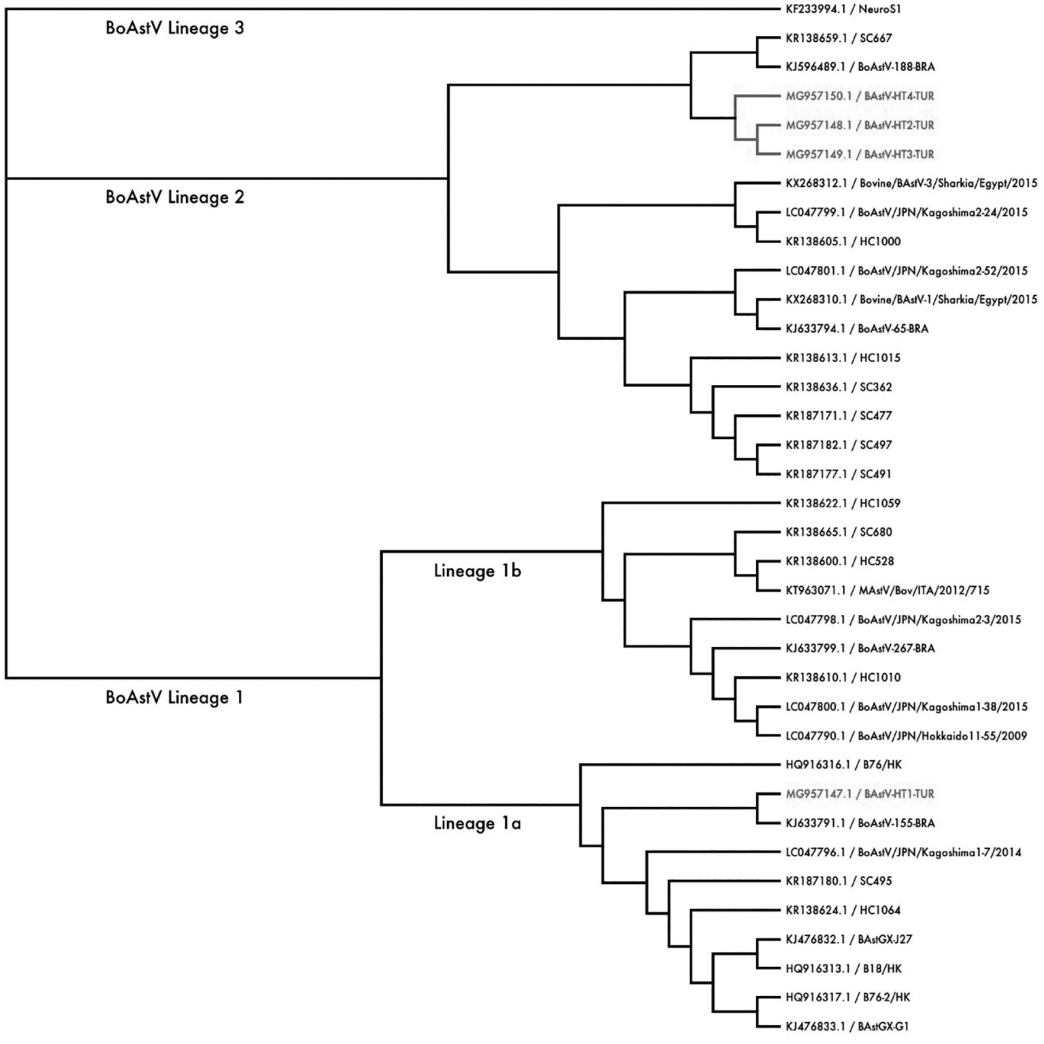
Results

According to molecular detection study based on nonstructural protein gene (Nsp1ab) of *Bovine astrovirus* using DPF/DPR primer set, which was redesigned for this study, four fecal samples (4/127, 3.15%) found to be positive. Partial sequences corresponding nonstructural protein gene were compared with other *Bovine astrovirus* sequence data which were online provided by National Center for Biotechnology Information (NCBI) (Table 2) by sequence alignment and phylogenetic analysis. While isolate BAstV/HT-1 was substituted under lineage 1a, BAstV/HT-2, 3 and 4 were substituted under lineage 2. None of novel BoAstV strains found to be related between the NeuroS1 which is a strain that isolated from a bovine with neurological symptoms.

Table 2. List of *Bovine astrovirus* sequences used in the molecular studies.

	GenBank Accession No	Strain/Isolate Name	Host	Year of Isolation	Country	Reference
1	KF233994.1	NeuroS1	Bovine	2011	USA	(20)
2	KJ596489.1	BoAstV-188-BRA	Bovine	2012	Brazil	(25)
3	KJ633790.1	BoAstV-43-BRA	Bovine	2007	Brazil	(25)
4	KJ633791.1	BoAstV-155-BRA	Bovine	2009	Brazil	(25)
5	KJ633794.1	BoAstV-65-BRA	Bovine	2007	Brazil	(25)
6	KJ633799.1	BoAstV-267-BRA	Bovine	2010	Brazil	(25)
7	KR187171.1	SC477	Bovine	2012	UK	(24)
8	KR187177.1	SC491	Bovine	2012	UK	(24)
9	KR187180.1	SC495	Bovine	2012	UK	(24)
10	KR187182.1	SC497	Bovine	2012	UK	(24)
11	KR138600.1	HC528	Bovine	2013	UK	(24)
12	KR138605.1	HC1000	Bovine	2013	UK	(24)
13	KR138610.1	HC1010	Bovine	2013	UK	(24)
14	KR138613.1	HC1015	Bovine	2013	UK	(24)
15	KR138622.1	HC1059	Bovine	2013	UK	(24)
16	KR138624.1	HC1064	Bovine	2013	UK	(24)
17	KR138636.1	SC362	Bovine	2012	UK	(24)
18	KR138659.1	SC667	Bovine	2013	UK	(24)
19	KR138665.1	SC680	Bovine	2013	UK	(24)
20	KJ476832.1	BAstGX-J27	Bovine	2013	China	(25)
21	KJ476833.1	BAstGX-G1	Bovine	2013	China	(25)
22	HQ916313.1	B18/HK	Bovine	2010	China	(10)
23	HQ916316.1	B76/HK	Bovine	2010	China	(10)
24	HQ916317.1	B76-2/HK	Bovine	2010	China	(10)
25	LC047790.1	BoAstV/JPN/Hokkaido11-55/2016	Bovine	2009	Japan	(15)
26	LC047796.1	BoAstV/JPN/Kagoshima1-7/2018	Bovine	2014	Japan	(15)
27	LC047798.1	BoAstV/JPN/Kagoshima2-3/2015	Bovine	2015	Japan	(15)

	GenBank Accession No	Strain/Isolate Name	Host	Year of Isolation	Country	Reference
28	LC047799.1	BoAstV/JPN/Kagoshima2-24/2015	Bovine	2015	Japan	(15)
29	LC047800.1	BoAstV/JPN/Kagoshima1-38/2017	Bovine	2015	Japan	(15)
30	LC047801.1	BoAstV/JPN/Kagoshima2-52/2015	Bovine	2015	Japan	(15)
31	KX268310.1	Bovine/BAstV-1/Sharkia/Egypt/2015	Bovine	2015	Egypt	(14)
32	KX268312.1	Bovine/BAstV-3/Sharkia/Egypt/2015	Bovine	2015	Egypt	(14)
33	KT963071.1	MAstV/Bov/ITA/2012/715	Bovine	2012	Italy	Unpublished
34	MG957147.1	BAstV-HT1-TUR	Bovine	2016	Turkey	(This study)
35	MG957148.1	BAstV-HT2-TUR	Bovine	2016	Turkey	(This study)
36	MG957149.1	BAstV-HT3-TUR	Bovine	2016	Turkey	(This study)
37	MG957150.1	BAstV-HT4-TUR	Bovine	2016	Turkey	(This study)



*Novel strains were illustrated as red color.

Figure 1. A phylogenetic bootstrap consensus tree of the partial Nsp1ab gene region of the *Bovine astrovirus* strains was made using the neighbor-joining method by using Unipro UGENE, version 1.21. [16].

Table 3. Sequence similarity chart of the *Bovine astrovirus* strains used in the study.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1 MG957147.1 / BAstV-HT1-TUR		76.8	76.8	75.8	90.4	82.9	81.4	83.3	82.1	79.5	82.7	84.1	77.4	70.9	69.3	72.7	72.7	72.2	71.4	71.3	72.7	73.2	77.3	80.0	80.0	80.0	79.5	78.2	77.8	79.1	78.0	75.9	76.4	55.2	76.3	75.8
2 MG957148.1 / BAstV-HT2-TUR	76.8		100.0	99.1	74.0	75.4	73.9	74.4	73.8	74.9	76.8	76.4	74.6	68.5	68.7	69.7	70.5	71.1	74.1	70.4	74.5	70.5	88.2	88.6	88.6	88.6	88.6	88.6	89.5	87.7	89.5	88.9	86.7	86.6	55.8	85.4	85.4
3 MG957149.1 / BAstV-HT3-TUR	76.8	100.0		99.1	74.0	75.4	73.9	74.4	73.8	74.9	76.8	76.4	74.6	68.5	68.7	69.7	70.5	71.1	74.1	70.4	74.5	70.5	88.2	88.6	88.6	88.6	88.6	88.6	89.5	87.7	89.5	88.9	86.7	86.6	55.8	85.4	85.4
4 MG957150.1 / BAstV-HT4-TUR	75.8	99.1	99.1		73.4	74.9	73.5	73.9	73.3	74.9	76.8	76.4	74.6	68.5	68.4	69.2	70.5	71.1	74.1	70.4	74.5	70.5	88.2	88.6	88.6	88.6	88.6	89.5	87.2	89.5	88.4	86.7	86.1	55.8	84.8	85.1
5 KJ633791.1 / BoAstV-155-BRA	90.4	74.0	74.0	73.4		82.0	80.5	83.4	81.4	78.7	81.6	82.7	77.8	68.9	69.6	70.7	68.7	71.0	70.4	69.8	72.6	72.6	77.1	79.9	79.9	79.9	78.2	79.9	76.6	81.0	75.4	74.3	73.1	53.9	72.9	74.2
6 KJ476832.1 / BAstGX-J27	82.9	75.4	75.4	74.9	82.0		92.5	88.3	88.4	83.4	84.1	87.3	75.5	69.6	69.0	71.0	69.5	72.2	70.9	70.7	71.7	72.3	73.6	75.9	77.3	77.3	76.8	77.7	76.6	79.1	74.8	74.5	73.0	54.5	72.0	73.6
7 HQ916313.1 / B18/HK	81.4	73.9	73.9	73.5	80.5	92.5		88.6	87.7	84.7	85.9	88.6	76.1	70.3	69.6	72.2	72.7	73.1	70.0	69.8	72.7	71.8	76.8	78.2	78.2	78.2	78.2	79.5	76.8	78.2	74.6	74.7	72.2	53.7	71.4	74.4
8 HQ916317.1 / B76-2/HK	83.3	74.4	74.4	73.9	83.4	88.3	88.6		90.8	84.5	84.1	88.6	76.1	69.1	69.0	71.0	69.1	74.7	70.9	71.2	70.0	71.8	75.5	79.1	79.1	79.1	78.6	78.2	77.5	79.5	75.1	74.5	72.2	55.8	73.2	72.8
9 KJ476833.1 / BAstGX-G1	82.1	73.8	73.8	73.3	81.4	88.4	87.7	90.8		85.3	83.2	86.4	77.8	69.8	69.6	72.0	69.5	72.7	70.0	71.3	70.5	72.7	76.8	79.1	79.1	79.1	77.7	79.1	77.1	80.5	76.8	74.4	74.2	55.2	73.8	74.7
10 LC047796.1 / BoAstV/JPN/Kagoshima1-7/2014	79.5	74.9	74.9	74.9	78.7	83.4	84.7	84.5	85.3		83.2	82.7	80.3	69.1	67.8	70.3	70.0	72.2	68.2	70.5	70.0	71.8	72.3	74.1	75.9	75.9	76.8	76.4	78.2	75.4	77.3	74.3	75.6	72.7	58.2	74.7	74.7
11 KR187180.1 / SC495	82.7	76.8	76.8	76.8	81.6	84.1	85.9	84.1	83.2	83.2		84.5	76.8	68.6	72.0	70.5	70.9	71.4	70.5	70.0	71.8	72.3	74.1	75.9	75.9	75.9	75.5	74.5	75.5	77.7	77.7	77.5	74.1	52.5	76.3	75.3
12 KR138624.1 / HC1064	84.1	76.4	76.4	76.4	82.7	87.3	88.6	88.6	86.4	82.7	84.5		76.4	68.2	68.5	71.4	71.8	71.8	70.9	71.8	72.3	70.5	76.8	77.3	77.3	77.3	76.8	78.2	80.9	78.6	78.2	78.2	75.0	54.3	77.4	77.3
13 HQ916316.1 / B76/HK	77.4	74.6	74.6	74.6	77.8	75.5	76.1	76.1	77.8	80.3	76.8	76.4		72.2	71.8	72.4	72.7	73.3	70.9	73.1	72.7	69.1	71.8	75.9	75.9	75.9	74.5	75.9	77.5	75.9	75.8	77.0	75.8	57.1	74.1	75.6
14 LC047800.1 / BoAstV/JPN/Kagoshima1-38/2015	70.9	68.5	68.5	68.5	68.9	69.6	70.3	69.1	69.8	69.1	68.6	68.2	72.2		89.0	90.7	89.5	87.5	84.1	84.0	83.6	69.5	73.6	69.1	69.1	69.1	68.6	69.5	72.6	70.0	70.3	68.0	67.9	57.3	68.2	70.5
15 KJ633799.1 / BoAstV-267-BRA	69.3	68.7	68.7	68.4	69.6	69.0	69.6	69.0	69.6	67.8	72.0	68.5	71.8	89.0		89.0	88.7	89.3	86.3	84.7	83.3	70.8	78.0	75.0	75.0	75.0	75.6	74.4	72.4	75.6	71.5	67.2	69.9	56.1	68.7	71.5
16 LC047790.1 / BoAstV/JPN/Hokkaido11-55/2009	72.7	69.7	69.7	69.2	70.7	71.0	72.2	71.0	72.0	70.3	70.5	71.4	72.4	90.7	89.0		90.5	90.7	85.9	86.5	86.4	70.9	75.0	71.4	71.4	71.4	70.9	71.4	73.5	71.4	70.0	69.4	71.2	57.1	68.2	72.2
17 KR138610.1 / HC1010	72.7	70.5	70.5	70.5	68.7	69.5	72.7	69.1	69.5	70.0	70.9	71.8	72.7	89.5	88.7	90.5		89.1	86.8	85.9	88.2	71.8	74.5	70.0	70.0	70.0	70.0	69.1	73.2	70.0	71.4	70.5	71.8	51.1	70.6	72.7
18 LC047798.1 / BoAstV/JPN/Kagoshima2-3/2015	72.2	71.1	71.1	71.1	71.0	72.2	73.1	74.7	72.7	72.2	71.4	71.8	73.3	87.5	89.3	90.7	89.1		88.2	86.3	85.5	71.8	78.2	73.6	73.6	73.6	74.1	74.5	73.1	75.5	70.8	71.0	69.7	57.6	68.8	71.1
19 KR138600.1 / HC528	71.4	74.1	74.1	74.1	70.4	70.9	70.0	70.9	70.0	68.2	70.5	70.9	70.9	84.1	86.3	85.9	86.8	88.2		91.8	90.0	73.2	78.2	70.9	70.9	70.9	70.9	71.8	75.5	74.5	75.0	73.6	70.9	52.9	73.4	75.3
20 KT963071.1 / MAstV/Bov/ITA/2012/715	71.3	70.4	70.4	69.8	71.7	69.8	71.2	71.3	70.5	70.0	71.8	72.3	73.1	84.0	84.7	86.5	85.9	86.3	91.8		90.5	69.5	75.9	71.8	71.8	71.8	70.9	72.7	71.9	73.6	71.5	70.1	68.4	58.9	69.3	70.5
21 KR138665.1 / SC680	72.7	74.5	74.5	72.6	72.3	72.7	70.0	70.5	70.0	71.8	72.3	72.7	83.6	83.3	86.4	88.2	85.5	90.0	90.5		70.9	79.1	68.6	72.7	72.7	72.7	71.8	75.9	73.6	76.8	75.0	73.2	51.1	74.0	76.3
22 KR138622.1 / HC1059	73.2	70.5	70.5	72.6	73.6	71.8	71.8	72.7	70.0	72.3	70.5	69.1	69.5	70.8	70.9	71.8	71.8	73.2	69.5	70.9		68.6	70.9	70.9	70.9	70.9	71.4	69.5	72.7	73.6	70.9	70.9	69.1	52.5	71.8	70.7
23 KR138659.1 / SC667	77.3	88.2	88.2	88.2	77.1	75.9	76.8	75.5	76.8	75.5	74.1	76.8	71.8	73.6	78.0	75.0	74.5	78.2	78.2	75.9	79.1	68.6		87.7	87.7	87.7	87.7	87.3	86.8	87.3	89.1	89.1	86.8	56.5	88.1	87.4
24 KR187182.1 / SC497	80.0	88.6	88.6	88.6	79.9	77.3	78.2	79.1	79.1	76.8	75.9	77.3	75.9	69.1	75.0	71.4	70.0	73.6	70.9	71.8	72.7	70.9	87.7		100.0	100.0	98.6	93.6	90.9	93.2	91.8	90.9	89.1	57.8	91.0	87.4
25 KR187177.1 / SC491	80.0	88.6	88.6	88.6	79.9	77.3	78.2	79.1	79.1	76.8	75.9	77.3	75.9	69.1	75.0	71.4	70.0	73.6	70.9	71.8	72.7	70.9	87.7	100.0		100.0	98.6	93.6	90.9	93.2	91.8	90.9	89.1	57.8	91.0	87.4
26 KR187171.1 / SC477	80.0	88.6	88.6	88.6	79.9	77.3	78.2	79.1	79.1	76.8	75.9	77.3	75.9	69.1	75.0	71.4	70.0	73.6	70.9	71.8	72.7	70.9	87.7	100.0	100.0		98.6	93.6	90.9	93.2	91.8	90.9	89.1	57.8	91.0	87.4
27 KR138636.1 / SC362	79.5	88.6	88.6	88.6	78.2	76.8	78.2	78.6	77.7	76.4	75.5	76.8	74.5	68.6	75.6	70.9	70.0	74.1	70.9	70.9	71.8	71.4	87.7	98.6	98.6	98.6		93.6	90.5	92.7	91.4	90.5	88.6	57.4	90.4	87.9
28 KR138613.1 / HC1015	78.2	89.5	89.5	89.5	79.9	77.7	79.5	78.2	79.1	78.2	74.5	78.2	75.9	69.5	74.4	71.4	69.1	74.5	71.8	72.7	71.8	69.5	87.3	93.6	93.6	93.6	93.6		91.8	92.7	91.4	91.4	89.5	57.4	91.0	88.4
29 LC047799.1 / BoAstV/JPN/Kagoshima2-24/2015	77.8	87.7	87.7	87.2	76.6	76.6	76.8	77.5	77.1	75.4	75.5	80.9	77.5	72.6	72.4	73.5	73.2	73.1	75.5	73.6	72.7	72.6	77.1	86.8	90.9	90.9	90.5	91.8		94.5	91.2	86.1	85.6	58.5	86.6	87.1
30 KR138605.1 / HC1000	79.1	89.5	89.5	89.5	81.0	79.1	78.2	79.5	80.5	77.3	77.7	78.6	75.9	70.0	75.6	71.4	70.0	75.5	74.5	73.6	73.6	73.6	87.3	93.2	93.2	93.2	92.7	92.7	94.5		94.1	91.8	88.2	58.7	89.8	87.9
31 KX268312.1 / Bovine/BAstV-3/Sharkia/Egypt/2015	78.0	88.9	88.9	88.4	75.4	74.8	74.6	75.1	76.8	74.3	77.7	78.2	75.8	70.3	71.5	70.0	71.4	70.8	75.0	71.5	76.8	70.9	89.1	91.8	91.8	91.8	91.4	91.4	91.2	94.1		88.9	87.6	55.3	87.5	87.1
32 LC047801.1 / BoAstV/JPN/Kagoshima2-52/2015	75.9	86.7	86.7	86.7	74.3	74.5	74.7	74.5	74.4	75.6	75.5	78.2	77.0	68.0	67.2	69.4	70.5	71.0	73.6	70.1	75.0	70.9	89.1	90.9	90.9	90.9	90.5	91.4	86.1	91.8	88.9		90.4	57.8	90.5	85.7
33 KX268310.1 / Bovine/BAstV-1/Sharkia/Egypt/2015	76.4	86.6	86.6	86.1	73.1	73.0	72.2	72.2	74.2	72.7	74.1	75.0	75.8	67.9	69.9	71.2	71.8	69.7	70.9	68.4	73.2	69.1	86.8	89.1	89.1	89.1	88.6	89.5	85.6	88.2	87.6	90.4		56.4	90.2	85.4
34 KF233994.1 / NeuroS1	55.2	55.8	55.8	55.8	53.9	54.5	53.7	55.8	55.2	58.2	52.5	54.3	57.1	57.3	56.1	57.1	51.1	57.6	52.9	58.9	51.1	52.5	56.5	57.8	57.8	57.8	57.4	57.4	58.5	58.7	55.3	57.8	56.4		57.4	53.8
35 KJ633794.1 / BoAstV-65-BRA	76.3	85.4	85.4	84.8	72.9	72.0	71.4	73.2	73.8	74.7	76.3	77.4	74.1	68.2	68.7	68.2	70.6	68.8	73.4	69.3	74.0	71.8	88.1	91.0	91.0	91.0	90.4	91.0	86.6	89.8	87.5	90.5	90.2	57.4		85.7
36 KJ596489.1 / BoAstV-188-BRA	75.8	85.4	85.4	85.1	74.2	73.6	74.4	72.8	74.7	74.7	75.3	77.3	75.6	70.5	71.5	72.2	72.7	71.1	75.3	70.5	76.3	70.7	87.4	87.4	87.4	87.4	87.9	88.4	87.1	87.9	87.1	85.7	85.4	53.8	85.7	

Discussion

The study reveals the first detection and phylogenetic analysis of BoAstV from diarrheic calves in Turkey. These fecal samples were previously studied for the presence of *Bovine coronavirus* (BCV), *Bovine rotavirus* (BRV) and *Bovine torovirus* (BToV) (unpublished), *Bovine kobuvirus* (BKV), *Bovine enterovirus* (BEV), *Bovine hungarovirus* (BHuV) (in press) , *Bovine norovirus* (BNoV) and *Bovine nebovirus* (BNeV) [26]. On the purpose of both detection and genetic characterization it was selected that 90 kDa non-structural protein, nsp1a, that contains a conserved protease motifs similar to other viral 3C-like proteases. Because of the diverse genetics of astroviruses, it is difficult to design precise detection tool by molecular basis. Multiple alignments of the BoAstV sequences, gathered from GenBank, indicated that it is necessity to check the detection primers by in-silico PCR method before using in a study. Thereby, we redesigned the forward primer of DPF as DPFr reported by Tse et. al., [25]. As a result of RT-PCR study, we found that the 3.15% of fecal samples (4/127) were positive for diarrhea calves from Turkey.

There are several reports on bovine astroviruses from healthy and diarrheic calves and the prevalences vary between 10 to 74 percent according to research papers worldwide. BoAstV were reported as 74% (85/115) from both healthy and diarrheic calves from Scotland [22]. Alfred and coworkers were reported the BoAstV as 46.10% from diarrheic calves of 3 different cattle farm in China in 2013 [1]. In another study reports the detection of BoAstV were 32% from diarrheic calves from 2 cattle farm in Egypt in 2015 [14]. On the other hand, paper published by Nagai et al., [15] reports the astrovirus positivity at 10.27% from calves with and without diarrhea in Japan between 2009 and 2015.

The neighbor-joining tree of the partial Nsp1ab gene (ORF1a) of bovine astroviruses indicated that strains were substituted under three distinct lineage. None of our strains belonged to the Neuro1 lineage (lineage 3) of bovine astroviruses which were isolated from bovine with neurological symptoms. On the other hand, while BAstV-HT1-TUR strain were classified under lineage 1a, the other novel starts were lineage 2 (Figure 1). Similar trees were illustrated by researchers previously based on both partially or complete sequences of BoAstV [1, 6, 14]. While the identity of analyzed sequences varies from 51.1 to 100%, novel strains were calculated between 75.8 to 100 % (Table 3).

As a result, to state the contribution of BoAstV on calf diarrhea more epidemiological study is needed. Also, definitive determination of bovine AstV as an enteric pathogen, either singly or in combination with another pathogen such as bovine rotavirus group A, would require viral isolation and experimental infections under natural conditions. We report the first detection and phylogenetic analysis of *Bovine astrovirus* from Turkey.

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