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ORIGINAL ARTICLE

Astroviruses and celiac disease: a preliminary study into potential environmental triggers

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ABSTRACT

Objective: Celiac disease (CD) is a chronic, multi-organ, autoimmune disease in which different viruses may play a role in the pathogenesis. human astroviruses (*Mamastrovirus*) have also been shown to infect enterocytes and replicate in intestinal enteroids. Therefore, astroviruses are thought to be one of the critical environmental factors for celiac patients. This is the the first study investigating relationship between CD and astroviruses.

Materials and Methods: Previously-described PCR protocols for screening and typing of mamastroviruses were modified and optimized. These molecular methods were used for the stool and duodenal biopsy samples of 53 patients; six newly diagnosed CD, three celiac patients with good treatment responses, 23 refractory CD and 21 patients with dyspepsia.

Results: Astroviruses could not be detected in the stool and duodenal biopsy samples of 53 patients.

Conclusion: Although no evidence for the association of *Mamastrovirus* infection and CD could be demonstrated in this study, this might have been due to limited cohort size. Therefore, comprehensive studies with larger samples with different patient groups are needed.

Keywords: celiac disease, human astrovirus, *Mamastovirus*, Türkiye

INTRODUCTION

Celiac disease (CD) is a chronic autoimmune/autoinflammatory disease with a lifelong gluten sensitivity of the small intestine. In the pathogenesis of CD, both innate and adaptive immune responses are stimulated by a mechanism that has not been fully elucidated. As a result of dysregulated immune response triggered by genetic and environmental factors, enterocytes of small intestine are damaged and malabsorption develops [1]. Viral infections are known to induce autoimmunity through different pathways and many autoimmune diseases are associated with viral infections [2,3]. Studies have shown that different viruses may also play role in the pathogenesis of celiac disease [2,4].

CD is a common disease affecting ~1% of most populations, and has an increasing prevalence observed in recent years [5,6]. The treatment for CD is based on the gluten free diet that restricts patient's life leading to uncompliance and suboptimal disease control. There is a need for better understanding the pathogenesis and developing novel treatment modalities. Not all genetic and environmental factors that influence the development and progression of the disease are recognized. Since a dysregulated immune response against gliadin causes damage to enterocytes, viruses have been investigated and shown to induce CD pathogenesis through molecular mimicry, immune activation, increased intestinal permeability and gut microbiome alterations [2,4,7-12]. Multiple studies have suggested that specific virus infections caused by *Rotavirus, Adenovirus, Reovirus,* and *Parechovirus* are linked to CD [7-12]. Although, viruses have been regarded to be one of the environmental factors that contribute to the disease's development, the mechanisms behind these relationships have remained unclear.

Mamastroviruses, one of the agents of viral gastroenteritis, have been reported to infect different tissues and organs other than the gastrointestinal tract and cause severe and systemic infections, especially in immunocompromised individuals [13]. They are non-enveloped, positive-sense, single-stranded RNA viruses have three genotypes/species were described; classic (HAstV-1–8, *Mamastrovirus hominis*), MLB (Melbourne) (MLB1-3, *Mamastrovirus melbournense*) and VA/HMO (Virginia/Human-Mink-Ovine-like) (VA1-5, *Mamastrovirus homustovis* and *Mamastrovirus virginiaense*) [14-20]. Although the capsid protein of astroviruses has the potential to damage the intestinal barrier, they do not induce clear pathology or cell death. There is little known about the mechanisms that influence the progression of mamastrovirus infections, hence it is unclear how other complications of infection, such as dysregulated secretion and malabsorption, emerge.

Experimental studies have demonstrated that mamastroviruses infect enterocytes and can replicate in intestinal enteroids, which are disease models generated by expanding patient-derived intestinal epithelial stem cells in 3D culture and used to study host-pathogen interactions [21]. If celiac patients' enterocytes are also infected with astroviruses, these viruses could be a initiating environmental factor in the pathogenesis of the disease by altering antigenic stimuli (via pathogen-recognizing molecular patterns (PAMP)), cytokine composition, and/or immune response in the microbiota. Mamastroviruses may be one of the critical environmental factors for celiac patients. This is a preliminary study aiming to determine whether there is a link between celiac disease and the presence of mamastroviruses.

MATERIALS AND METHODS

Sampling

The study was designed as a prospective cross sectional study, and was approved by the Hacettepe University Non-Invasive Clinical Research Ethics Committee (No: 2020/20-29 and 2021/13-125).

The study included four groups; namely patients with newly diagnosed CD, good treatment responsive CD, inadequate treatment response, and dyspepsia who were on follow up at Gastroenterology Clinic in Hacettepe University Hospital between April 2021 and March 2023. The duodenal biopsies and stool samples were collected and tested for the presence of astroviruses via reverse-transcription PCR (RT-PCR).

Nucleic acid isolation and screening

Nucleic acids were extracted from duodenal biopsies and stool samples using NucleoSpin RNA virus kit (Macherey-Nagel, Germany). RNA was reverse transcribed with random hexamers using the instructions of manufacturer (A.B.T. cDNA Synthesis Kit with RNAse Inhibitor (C03-01-20), A.B.T Laboratory Industry, Türkiye).

The 430base pair (bp) sequence in the RNA-dependent RNA polymerase encoding region was amplified via the semi-nested PCR. The primers provided by Japhet et al. and Finkbeiner et al. were revised and used in this study (Table 1) [18,22].

Each reaction mixture contained 2 mM $MgCl_2$, 0.3 mM dNTPs, 10 pmol of primers, and 0.75 U Taq DNA polymerase (A.B.T Laboratory Industry, Türkiye) in 30 µL volume. Thermal cycling parameters for the first round were as follows: an initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 51°C for 1 min, extension at 72°C for 1 min and the final extension step of 72°C for 3 min. An initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 53°C for 30 s, extension at 72°C for 1 min, and a final extension step of 72°C for 3 min for the second round. PCR products were visualized by electrophoresis on 1.5% agarose gels.

The methods were validated using stool sample from a patient infected with *Mamastrovirus* infection. The sample was used in each extraction process, whereas isolated RNA was used in each reverse transcription and PCR run.

Mamastrovirus typing

Different PCR protocols were applied for the typing of mamastroviruses as classical (*Mamastrovirus hominis*), MLB (*Mamastrovirus melbournense*) and VA/HMO (*Mamastrovirus homustovis* and *Mamastrovirus virginiaense*). Each reaction mixture contained 2 mM $MgCl_2$, 0.3 mM dNTPs, 10 pmol of primers (Table 2), and 0.75 U Taq DNA polymerase (A.B.T Laboratory Industry, Türkiye) in 30 µL volume. Thermal cycling parameters were indicated below for each type and PCR products were visualized by electrophoresis on 1.5% agarose gels.

Table 1. Primers used for the screening of samples

Primers	Polarity	Sequence	Reference
AV93 (s)-1	Sense	GAYTGGACICGNTWTGATGG	Revised from Japhet et al. [22]
AV91 (as)-1	Antisense	TTTGGTCCDCCCCTCCA	Revised from Japhet et al. [22]
SF0076 (as)	Antisense	CWGGYTTDACCCACATNCC	Revised from Finkbeiner et al. [18]

Table 2. Primers used for the typing of astroviruses

Type	Primer	Polarity	Sequence	Reference
Classical (*Mamastrovirus hominis*)	Mon269-1	Sense	CAACTCAGGAAACARGGTGT	Revised from Finkbeiner et al. [18]
	AstVcR	Antisense	GCATANCCTGTRAANCACCA	This study
MLB (*Mamastrovirus melbournense*)	SF0053	Sense	CTGTAGCTCGTGTTAGTCTTAACA	Finkbeiner et al. [18]
	SF0061-1	Antisense	GTTCATTRGCACCATCAGARC	Revised from Finkbeiner et al. [18]
VA/HMO (*Mamastrovirus homustovis* and *Mamastrovirus virginiaense*)	SF0178-1	Sense	GCTGTMACCGTCTCTGCCACCAT	Revised from Finkbeiner et al. [18]
	SF0179-1	Antisense	CATGCTGCATCCTTGTAGGTAGA	Revised from Finkbeiner et al. [18]

Classical mamastroviruses (*Mamastrovirus hominis*); an initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 45 s, extension at 72°C for 1 min and the final extension step of 72°C for 3 min. Product size is 575 bp.

MLB mamastroviruses (*Mamastrovirus melbournense*); an initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 1 min and the final extension step of 72°C for 3 min. Product size is 402 bp.

VA/HMO mamastroviruses (*Mamastrovirus homustovis* and *Mamastrovirus virginiaense*); an initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 1 min and the final extension step of 72°C for 3 min. Product size is 475 bp.

RESULTS

Demographic characteristics of the patients

The duodenal biopsies and stool samples of 53 patients were included in this study. There were six patients with newly diagnosed CD, three CD patients with well treatment response and 23 CD patients with inadequate treatment response, and 21 patients with dyspepsia. Thirty four of the patients (64%) were women, and 19 (36%) of them were men. The mean age was 39 years.

Molecular screening and typing of samples

Protocols for screening and typing of samples were optimized using a positive control samples (Figure 1 and 2). We screened duodenal biopsies and stool samples belonging to 53 patients, however mamastroviruses were not detected in these samples (Figure 3).

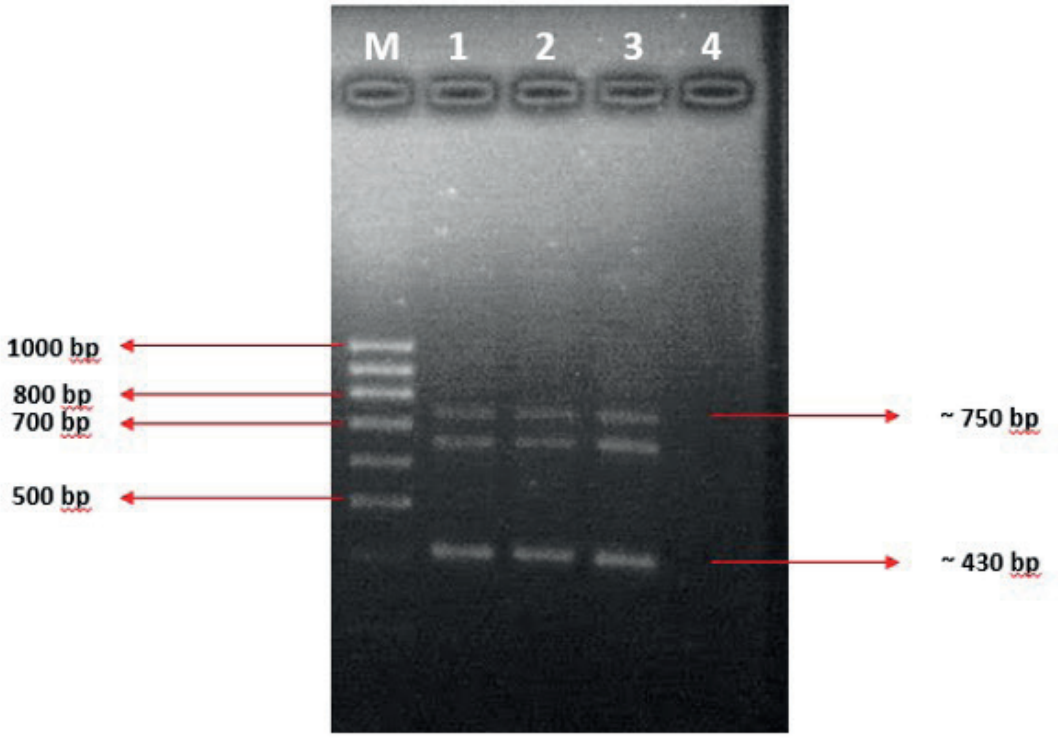

Figure 1. Optimization results of semi-nested PCR protocol used for screening. The bands belong to the positive control. The expected product size is 750 bp for RT-PCR and ~430 bp for semi-nested PCR. Wells 1, 2 and 3 belong to the positive control, whereas well 4 belongs to the negative control. M: Marker (100-1000 bp).

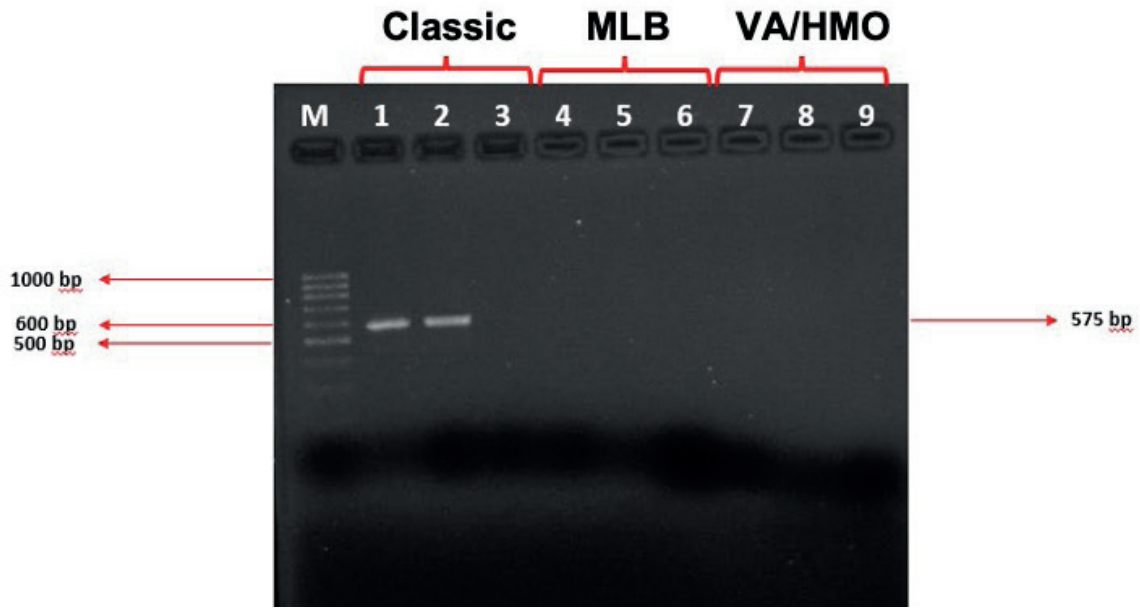

Figure 2. Optimization results of PCR protocols used for typing. The bands belong to the positive control. Wells 1 and 2 are the classic astroviruses (HAstV-1–8, *Mamastrovirus hominis*) (575 bp) and well 3 is the negative control. Wells 4 and 5 are the PCR result specific for the MLB astroviruses (MLB1-3, *Mamastrovirus melbournense*) (402 bp) and well 6 is the negative control. Wells 7 and 8 are VA/HMO astroviruses (VA1-5, *Mamastrovirus homustovis* and *Mamastrovirus virginiaense*) specific PCR result (475 bp) and well 9 is negative control. M: Marker (100-1000 bp).

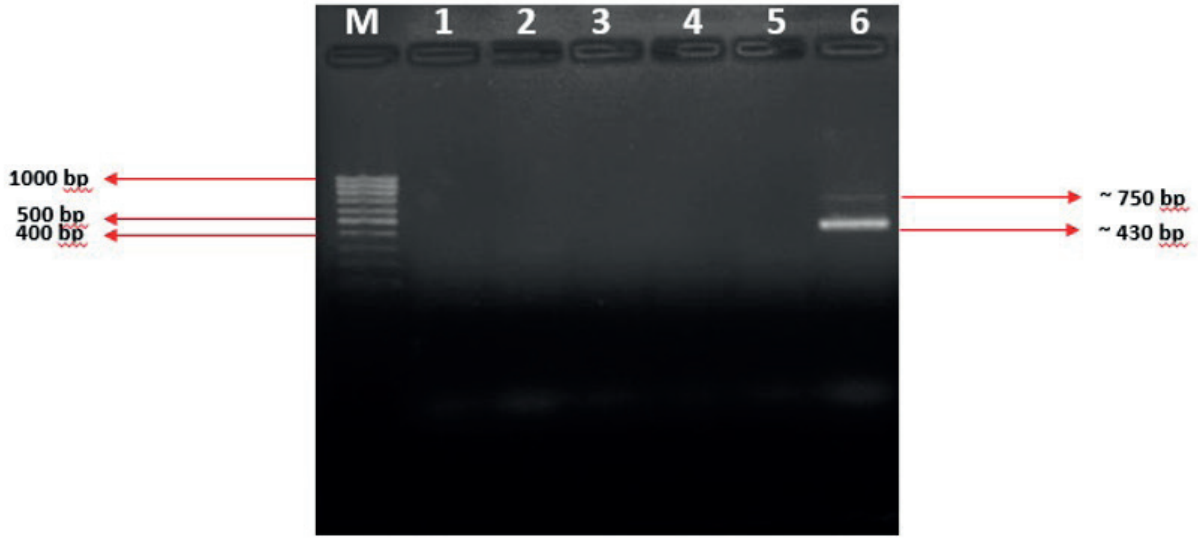

Figure 3. Screening PCR results of patient samples. Wells 1, 2, 3 and 4 are patient samples, well 5 is negative control and well 6 is positive control. M: Marker (100-1000 bp).

DISCUSSION

CD is a multisystemic disease characterized by enterocyte damage caused by gluten consumption and the development of specific antibodies. Several studies have established a link between various enteric viruses and the pathogenesis of celiac disease [4,8-12]. However, the relationship between mamastroviruses, which are known to cause gastroenteritis—particularly in immunocompromised individuals and children—and celiac disease remains largely unexplored [23,24]. Additionally, there is limited information regarding the prevalence of mamastroviruses in Turkey [24]. This study aims to serve as a preliminary investigation into whether there is an association between CD and the presence of mamastroviruses.

Stool and duodenal biopsy samples of 53 patients, including newly diagnosed celiac disease patients, celiac patients with good and inadequate response

to treatment and dyspepsia patients, were screened using PCR-based methods but no evidence for mamastroviruses were documented.

It is important to note that the study faced particular limitations that may have influenced the results. Primary limitation was the sample size, which may not have been large enough to detect low-frequency viral infections. The study was conducted during the COVID-19 pandemic, many of the follow-up patients postponed their follow-up visits, making it unable to reach the targeted patient population in each group. It is possible that larger, more comprehensive studies with a greater number of patients—particularly those representing various stages of CD, including those with different treatment responses—would yield different results.

The study did not find any mamastroviruses in the patient samples while it is important to acknowledge that the pathogenesis of CD is multifactorial. Although viral infections, including mamastroviruses, could potentially act as environmental triggers, the immune response to gluten and the genetic predisposition of individuals remain the primary drivers of the disease.

In conclusion, while our findings do not support a direct link between mamastroviruses and celiac disease, they underscore the need for further research to explore the complex interactions between viral infections and autoimmune disorders like CD. To definitively clarify whether mamastroviruses play a role in the pathogenesis of CD, future studies should aim to include larger and more diverse patient populations, encompassing a broader range of CD subtypes.

Author contribution

Study conception and design: CP, HYB, and KE; data collection: CP, TŞ, CŞ, İET, Sİ, CS, and HŞ; analysis and interpretation of results: CP, TŞ, and İET; draft manuscript preparation: CP, HYB, and KE. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

The study was approved by the Hacettepe University Non-Invasive Clinical Research Ethics Committee (Protocol no. 2020/20-29 and 2021/13-125).

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] Rosenbaum JT. Celiac disease and autoimmunity - the missing ingredient. N Engl J Med 2017;377(15):1489-90. https://doi.org/10.1056/NEJMcibr1706917

[2] Lerner A, Arleevskaya M, Schmiedl A, Matthias T. Microbes and viruses are bugging the gut in celiac disease. Are they friends or foes? Front Microbiol 2017;8:1392. https://doi.org/10.3389/fmicb.2017.01392

[3] Smatti MK, Cyprian FS, Nasrallah GK, Al Thani AA, Almishal RO, Yassine HM. Viruses and autoimmunity: A review on the potential interaction and molecular mechanisms. Viruses 2019;11(8):762. https://doi.org/10.3390/v11080762

[4] Bouziat R, Hinterleitner R, Brown JJ, et al. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. Science 2017;356(6333):44-50. https://doi.org/10.1126/science.aah5298

[5] Lebwohl B, Rubio-Tapia A. Epidemiology, presentation, and diagnosis of celiac disease. Gastroenterology 2021;160(1):63-75. https://doi.org/10.1053/j.gastro.2020.06.098

[6] King JA, Jeong J, Underwood FE, et al. Incidence of celiac disease is increasing over time: A systematic review and meta-analysis. Am J Gastroenterol 2020;115(4):507-25. https://doi.org/10.14309/ajg.0000000000000523

[7] Størdal K, Kahrs C, Tapia G, Agardh D, Kurppa K, Stene LC. Review article: Exposure to microbes and risk of coeliac disease. Aliment Pharmacol Ther 2021;53(1):43-62. https://doi.org/10.1111/apt.16161

[8] Stene LC, Honeyman MC, Hoffenberg EJ, et al. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: A longitudinal study. Am J Gastroenterol 2006;101(10):2333-40. https://doi.org/10.1111/j.1572-0241.2006.00741.x

[9] Perfetti V, Baldanti F, Lenti MV, et al. Detection of active epstein-barr virus infection in duodenal mucosa of patients with refractory celiac disease. Clin Gastroenterol Hepatol 2016;14(8):1216-20. https://doi.org/10.1016/j.cgh.2016.03.022

[10] Kahrs CR, Chuda K, Tapia G, et al. Enterovirus as trigger of coeliac disease: Nested case-control study within prospective birth cohort. BMJ 2019;364:l231. https://doi.org/10.1136/bmj.l231

[11] Kagnoff MF, Austin RK, Hubert JJ, Bernardin JE, Kasarda DD. Possible role for a human adenovirus in the pathogenesis of celiac disease. J Exp Med 1984;160(5):1544-57. https://doi.org/10.1084/jem.160.5.1544

[12] Tapia G, Chudá K, Kahrs CR, et al. Parechovirus infection in early childhood and association with subsequent celiac disease. Am J Gastroenterol 2021;116(4):788-95. https://doi.org/10.14309/ajg.0000000000001003

[13] Vu DL, Cordey S, Brito F, Kaiser L. Novel human astroviruses: Novel human diseases? J Clin Virol 2016;82:56-63. https://doi.org/10.1016/j.jcv.2016.07.004

[14] International Committee on Taxonomy of Viruses (ICTV). Virus taxonomy: The classification and nomenclature of viruses, The 9th report of the ICTV. San Diego; Elsevier; 2012.

[15] Finkbeiner SR, Allred AF, Tarr PI, Klein EJ, Kirkwood CD, Wang D. Metagenomic analysis of human diarrhea: Viral detection and discovery. PLoS Pathog 2008;4(2):e1000011. https://doi.org/10.1371/journal.ppat.1000011

[16] Finkbeiner SR, Kirkwood CD, Wang D. Complete genome sequence of a highly divergent astrovirus isolated from a child with acute diarrhea. Virol J 2008;5:117. https://doi.org/10.1186/1743-422X-5-117

[17] Finkbeiner SR, Li Y, Ruone S, et al. Identification of a novel astrovirus (astrovirus VA1) associated with an outbreak of acute gastroenteritis. J Virol 2009;83(20):10836-9. https://doi.org/10.1128/JVI.00998-09

[18] Finkbeiner SR, Holtz LR, Jiang Y, et al. Human stool contains a previously unrecognized diversity of novel astroviruses. Virol J 2009;6:161. https://doi.org/10.1186/1743-422X-6-161

[19] Finkbeiner SR, Le BM, Holtz LR, Storch GA, Wang D. Detection of newly described astrovirus MLB1 in stool samples from children. Emerg Infect Dis 2009;15(3):441-4. https://doi.org/10.3201/eid1503.081213

[20] Kapoor A, Li L, Victoria J, et al. Multiple novel astrovirus species in human stool. J Gen Virol 2009;90(Pt 12):2965-72. https://doi.org/10.1099/vir.0.014449-0

[21] Kolawole AO, Mirabelli C, Hill DR, et al. Astrovirus replication in human intestinal enteroids reveals multi-cellular tropism and an intricate host innate immune landscape. PLoS Pathog 2019;15(10):e1008057. https://doi.org/10.1371/journal.ppat.1008057

[22] Japhet MO, Famurewa O, Adesina OA, et al. Viral gastroenteritis among children of 0-5 years in Nigeria: Characterization of the first Nigerian aichivirus, recombinant noroviruses and detection of a zoonotic astrovirus. J Clin Virol 2019;111:4-11. https://doi.org/10.1016/j.jcv.2018.12.004

[23] Daniel-Wayman S, Fahle G, Palmore T, Green KY, Prevots DR. Norovirus, astrovirus, and sapovirus among immunocompromised patients at a tertiary care research hospital. Diagn Microbiol Infect Dis 2018;92(2):143-6. https://doi.org/10.1016/j.diagmicrobio.2018.05.017

[24] Aktaş O, Aydin H, Timurkan MO. A molecular study on the prevalence and coinfections of Rotavirus, Norovirus, Astrovirus and Adenovirus in children with gastroenteritis. Minerva Pediatr 2019;71(5):431-7. https://doi.org/10.23736/S0026-4946.16.04304-X