BMC Infectious Diseases

RESEARCH

Open Access

Epidemiology and genetic diversity of classic human astrovirus with whole-genome sequence analysis in inpatient children under 5 years of age in Yunnan, China

Zhichao Wang[1†], Lili Jiang[1†], Lihua Chen[1†], Jianping Cun[1], Xiaofang Zhou[1], Yongming Zhou[1], Wenpeng Gu[1*] and Yihui Cao[1*]

Abstract

Purpose The aim of this study was to determine the epidemiology, incidence and genetic diversity of classic human astrovirus (HAstV) in inpatient children under 5 years of age for acute gastroenteritis (AGE).

Methods A hospital-based surveillance study was conducted across Yunnan Province to investigate the incidence of HAstV among AGE patients. Viral RNA was extracted from stool samples collected from January 2015 to December 2023 from hospitalized children under 5 years of age with AGE. Demographic and clinical data were collected and analysed. The RNA of eligible stool samples ($n=2501$) was screened via real-time RT–PCR assays for the presence of classical HAstV via a real-time PCR diagnostic kit for rapid detection of HAstV (XABT, China). The positive HAstV samples (Ct < 25) were subjected to next-generation sequencing (NGS), and phylogenetic analysis was performed for genotypic characterization.

Results A total of 2501 stool samples from hospitalized children < 5 years old were analysed for the presence of classic HAstV from 2015 to 2023. HAstV RNA was detected in 4.88% (122/2501) of the stool samples in the study. There were 1.46 times more male patients than female patients (1484/1017), and their HAstV detection rates were 4.58% (68/1484) and 5.31% (54/1017), respectively, with no statistically significant difference ($\chi^2=0.688$ $P=0.407$). Among the patients, the average age was 12 ± 17 (monthly age, $M \pm Q$). Children between 2 and 4 years of age were more affected by HAstVs, while the highest positivity detection rate was found in the 24–35 month age group (7.56%, 17/225). Interestingly, the detection rate of HAstV in 2019 was 17.79% (45/253), which was significantly higher than that in other years during the surveillance period ($\chi^2=126.229$, $P<0.05$). The data revealed that the prevalence of HAstV was greater in the summer (10.04%, 50/498) than that in the other seasons in Yunnan. Seventeen HAstV strains

†Zhichao Wang, Lili Jiang and Lihua Chen contributed equally to this work.

*Correspondence:
Wenpeng Gu
gu_02788@163.com
Yihui Cao
526505779@qq.com

Full list of author information is available at the end of the article

were sequenced. Further analysis of the complete genome and phylogenetic analysis revealed the presence of classic HAstV-1 – HAstV-4 genotypes. HAstV-1 was the most commonly detected genotype, and all 11 HAstV-1 strains were classified to 1a subtype.

Conclusion This study provided valuable insights into the epidemiology and genotype diversity of HAstVs in hospitalized children under 5 years of age with AGE. The results can be used in future preventive measurements and the development of effective vaccines.

Keywords Astrovirus, Children, Acute gastroenteritis, Genotypes, Hospitalizations

Introduction

In 1975, human astrovirus (HAstV) was initially discovered in humans [1,2]. To date, HAstV is a well-established significant agent of acute gastroenteritis (AGE) with a worldwide distribution among young children [3–5] and represents one of the most common viruses associated with AGE [6, 7]. HAstV belongs to the *Astroviridae* family, which encapsulates a nonsegmented, positive-sense, single-stranded RNA genome into ~30 nm icosahedral particles with no envelope. The genome is ~7 kb in length and contains three open reading frames (ORF1a, ORF1b and ORF2) [8]. ORF2 encodes a capsid protein, according to which HAstVs are classified into three clades: classic HAstV, Melbourne (MLB), and Virginia (VA)[9, 10]. Classic HAstVs are important causative agents of sporadic diarrhea and outbreaks of AGE in children and can also be associated with neurological syndromes and encephalitis in vulnerable individuals [11–13]. There are eight distinct serotypes (HAstV-1–HAstV-8) [14]. According to Martella [15] and Bosch [3], HAstV-1 can be classified into six lineages (1a to 1f): HAstV-2 (2a to 2d), HAstV-3 (3a and 3b), HAstV-4 (4a to 4c), HAstV-5 (5a to 5c), and HAstV-6 (6a and 6b). The prevalence of HAstVs among children varies from 0 to over 20% globally with a heavy disease burden [10, 16, 17–19]. Although young children are most threatened by HAstVs, limited information is available in Yunnan. In particular, systematic epidemiological studies to determine their true prevalence and genetic diversity are still lacking. In this study, nine continuous years of surveillance was carried out to investigate the epidemiology and divergence of classic HAstV in pediatric hospitalized patients with AGE, hoping to provide references for disease control and prevention.

The study was conducted in accordance with the Declaration of Helsinki, and ethical approval was given by the Ethical Review Committee of the Yunnan Center for Disease Control and Prevention. Informed consent was obtained from the parent of each child.

Materials and methods

Clinical stool samples

According to the National Surveillance Protocol for Viral Diarrhea (2021 version), AGEs are defined as the occurrence of three or more episodes of loose stool or looser-than-normal stool within 24 h, including a watery or thin paste texture and the presence of mucous or < 3 stools per day with abnormal stools possibly accompanied by vomiting, abdominal pain, fever, nausea, and dehydration. In total, 2051 stool samples from hospitalized patients under 5 years of age with AGE from January 2015 to December 2023 were collected and retrospectively analysed for classic HAstVs. All the enrolled stool samples were derived from residual samples obtained after routine testing and were initially stored at − 70 °C when collected from the inpatient department before undergoing classic HAstV detection.

RNA extraction and HAstV detection

HAstV RNA was extracted from 200 µl of 10% supernatant with the Viral Nucleic Acid Isolation Kit (BioPerfectus, China, SDK60104) according to the manufacturer's instructions [20]. Classic HAstV was identified via a real-time PCR diagnostic kit for rapid detection of astrovirus (XABT, China) in accordance with the instructions. Astrovirus-specific real-time PCRs of the capsid gene were performed in a 96-well reaction plate under the following conditions: 50 °C for 10 min, 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. Ct values < 38 were considered to be positive, and all the positive samples Ct values were recorded. All the positive HAstV samples (Ct < 25) were sent to Shanghai Bojie Biotechnology Co., Ltd., for next-generation sequencing (NGS).

Next-generation sequencing

The nucleic acids of the HAstV-positive samples (Ct < 25) were captured with the HAstV genome enrichment kit of Shanghai Bojie Biotechnology Co., Ltd., and the amplified PCR products were purified and quantified and then subjected to deep sequencing via Illumina MiSeq with 2 × 300 base paired-end runs with an Illumina Nextera XT Kit. The Q30 of the sequencing data was > 85%, and the data were analysed via the CLC Genomics Workbench 12 (Qiagen, Germany). More than 90% of the sequencing reads reached the Q30 (99.9% base call accuracy). The quality of the sequenced reads was assessed via FastQC. The sequencing data volume of each sample was

Table 1 Demographic characteristics of children with classic HAstV in Yunnan, 2015–2023

Epidemiological data	No. of HAstV positive samples ($n=122$)	samples analysed ($n=2501$)	Infection rate (%)	χ^2 and P value
Gender				
Male	68	1484	4.58	$\chi^2=0.688^*P=0.407$
Female	54	1017	5.31	
Age(months)				
<0–5	26	649	4.01	$\chi^2=6.064^*P=0.300$
6–11	33	685	4.82	
12–23	33	658	5.02	
24–35	17	225	7.56	
36–47	10	172	5.81	
48–59	3	112	2.68	
Season				
Spring	31	624	4.97	$\chi^2=39.859^*$ $P<0.05$
Summer	50	498	10.04	
Autumn	16	452	3.54	
Winter	25	927	2.70	

Notes: *chi-square test

1 Gb, with 22–33 million reads. The reads and average sequencing depth of the equilibrium distribution of each base were approximately 600x, and the sequencing depth was greater than 30x, with a comparison rate greater than 99.99%.

Sequence analysis

A phylogenetic tree for the complete genome was constructed with MEGA 10.0 software via the neighbor–joining method, with 1,000 bootstrap replications based on the Kimura two-parameter model. The reference strains from GenBank representing different HAstV genotypes were included in the phylogenetic analysis. The evolutionary distances were computed via the p-distance method. The complete genome sequences of HAstV described in this study were deposited in the GenBank database with the accession numbers PQ510853–PQ510867 and PQ496890–PQ496891.

Statistical analysis

The data were analysed with SPSS 26. Comparisons were performed via chi-square test. The differences between patients with positive and negative HAstV results were compared. Variable differences were considered significant if the p value was <0.05.

Results

Demographic characteristics

A total of 2501 stool samples were analysed for the presence of HAstV from 2015 to 2023. A total of 59.33% (1484/2501) of the samples were from male patients, and 40.66% (1017/2501) were from female patients (Table 1). HAstV RNA was detected in 4.88% (122/2501) of the stool samples in the present study. There were 1.46 times more male patients than female patients, and the

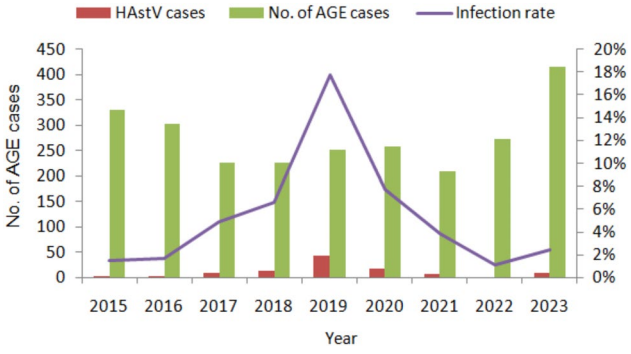

Fig. 1 AGE cases and HAstV-positive detection rates from 2015–2023. From 2015–2023, the positive detection rate of HAstV continued to rise from 2015–2019 and peaked in 2019 (17.79%; 45/253), with a significant difference (chi-square test, $\chi^2=126.229$, $P<0.05$); after that, it began to decline

HAstV detection rates were 4.58% (68/1484) and 5.31% (54/1017), respectively. A higher positivity detection rate was found in females than in males, but this difference was not statistically significant ($\chi^2=0.688$, $P=0.407$). To analyse the prevalence of HAstV infection on the basis of demographic characteristics, the patients were stratified into six different age groups. Among the patient characteristics, the average age was 12 ± 17 (monthly age, $M\pm Q$), and children between 2 and 4 years of age were most affected by HAstV, while the highest positivity detection rate was found in the 24–35 month age group (7.56%, 17/225). Interestingly, the detection rate of HAstV in 2019 was 17.79% (45/253) (Fig. 1), which was significantly higher than that in other years during the surveillance period ($\chi^2=126.229$, $P<0.05$). The data revealed that the prevalence of classic HAstV was highest in the summer at 10.04% (50/498) in Yunnan Province ($\chi^2=39.859$, $P<0.05$).

Phylogenetic analysis

HAstVs in the samples likely decreased after long-term storage in this study, and only 17 complete genome sequences were obtained from 2022 to 2023. Further NGS analysis of the complete genome and phylogenetic analysis revealed the presence of HAstV-1 to HAstV-4 genotypes circulating in Yunnan. For phylogenetic analysis, reference sequences for HAstVs were selected considering the standards of each genetic type of HAstV, and other similar sequences representing different geographical regions were also used. Analysis of the sequences generated from the samples revealed that 11 (64.70%; 11/17) strains were phylogenetically related to classical HAstV-1, 4 (23.53%; 4/17) were HAstV-4, and the remaining 2 strains included 1 HAstV-2 strain and 1 HAstV-3 strain (Fig. 2). HAstV-1 was the dominant genotype. After further analysis, all 11 classic HAstV-1 strains in Yunnan Province were reclassified into the 1a subtype (Fig. 3). The similarity of nucleotides and amino acids among all the HAstVs was 70.20-99.99% and 70.80-100%, respectively. In different regions, the HAstVs genotypes varied. Of the17 HAstVs, HAstV-1 was mainly prevalent in Baoshan, while the predominant strain in Xishuangbanna was the HAstV-4 (Fig. 4).

Figure 4 in Baoshan, there were 13 strains were sequenced with 3 genotypes accounting for 76.46%; eleven HAstV-1 stains, one HAstV-2 and 1 HAstV-3. HAstV-4 strains were detected in Xishuangbanna (3; 17.65%) and Yuxi (1; 5.88%)

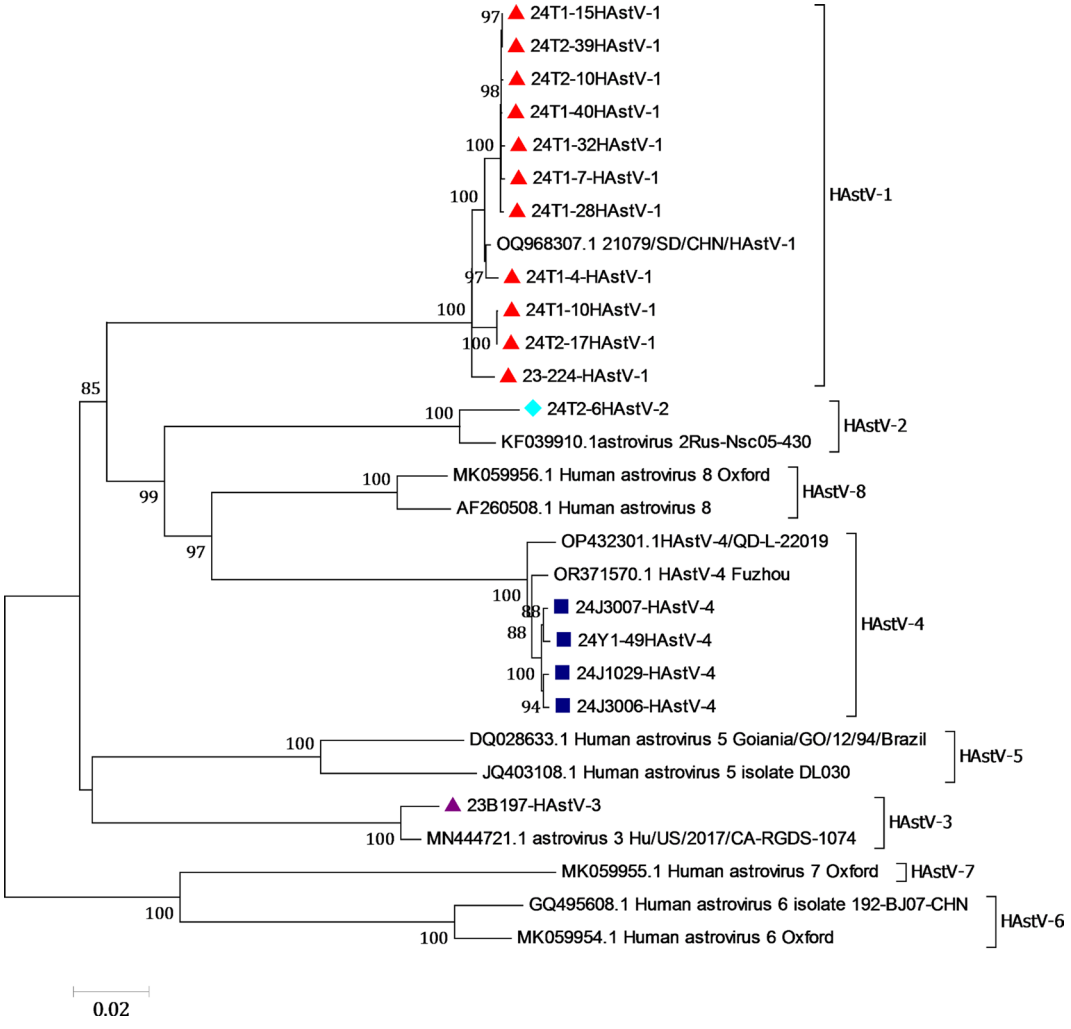

Fig. 2 Phylogenetic tree based on the whole sequences of classic HAstVs via the neighbor–joining method. The red triangle (▲) indicates HAstV-1, and the deep blue square (■) indicates HAstV-4. (◆) and (▲) indicate HAstV-2 and HAstV-3, respectively. A total of 12 reference nucleotide sequences were randomly selected from GenBank, with their respective accession numbers shown in the tree. The evolutionary distances were computed via the p-distance method and expressed in units of the number of base differences per site. Evolutionary analyses were conducted in MEGA X (10) and bootstrap tests (1000 replicates) on the basis of the Kimura two-parameter model

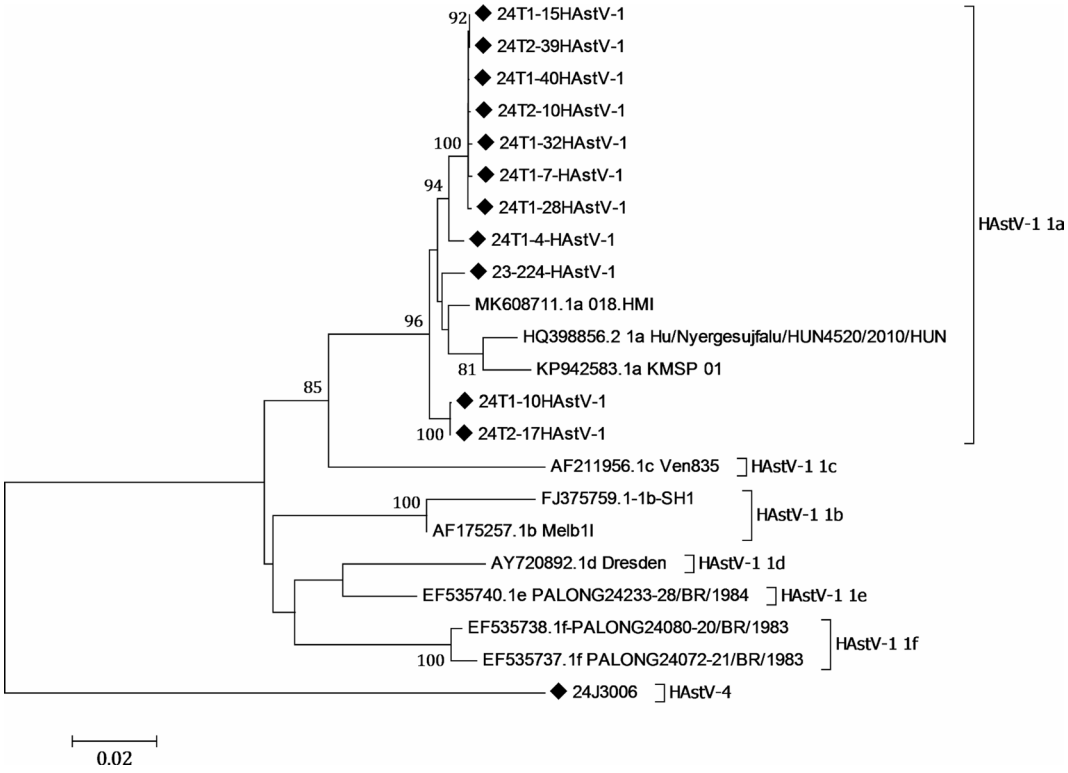

Fig. 3 Phylogenetic analysis of classic HAstV-1 strains in Yunnan Province All 11 classic HAstV-1 strains in Yunnan Province were reclassified into the 1a subtype via the neighbor–joining method. (◆) Yunnan HAstV strains. The evolutionary distances were computed via the p-distance method and expressed in units of the number of base differences per site with MEGA X (10) and bootstrap tests (1000 replicates) on the basis of the Kimura two-parameter model

Discussion

With improvements in sanitation and safe water supplies, the occurrence of bacterial AGEs has declined remarkably, and viruses have become the leading causes of AGE [21, 22]. In 1975, the first classic HAstV was discovered. To date, classic HAstV has been associated with gastrointestinal illness globally [4, 7, 16, 23] and has gained worldwide attention after rotavirus and norovirus [10], especially in young [24–26], elderly [27], and immunocompromised people [4]. The increased severity of clinical symptoms and extragastrointestinal involvement associated with HAstV infection have drawn much attention [28]. In this study, the prevalence rate of HAstV was not influenced by gender [24]. HAstV RNA was detected in 4.88% of the stool samples. The HAstV detection rate in male patients was 4.58%, and that in female patients was 5.31%, with no statistically significant difference ($\chi 2 = 0.688$, $P = 0.407$). Children of all ages were affected by HAstVs [24,viral gastroenteritis 29]. We found that children with AGE aged 2–4 years were more susceptible to infection, especially those aged 24–35 months.

This study demonstrated a clear seasonal distribution of classic HAstV over a nine-year period, with a more pronounced prevalence occurring mainly during the warm season, with peaks in summer. In Korea, a surge in HAstV infection in summer was also observed [30]. In Chongqing, China, HAstV infection shared the same seasonal pattern [31]. The prevalence of HAstV was 10.04%, which was significantly different ($\chi^2 = 39.859$, $P < 0.05$).

Interestingly, the positive detection rate of HAstV in 2019 was 17.79% (45/253), which was significantly higher than that in other years during the surveillance period. Unique geographical and excellent climatic conditions attracted many people to travel to Yunnan. Before COVID-19, the rapid development of the Chinese economy drove Yunnan's tourism, which likely increased the risk of HAstV infection. From 2015 to 2019, the detection rate of HAstVs in Yunnan Province increased. During the high incidence of COVID-19 from 2020 to 2022 across the whole country, Chinese people took many measures against the epidemic, such as mask wearing, lockdowns, physical distancing, and school suspensions, which indirectly suppressed the spread of HAstVs.

The predominant genotype varies with time and location [618]. However, limited information about HAstVs is available in Yunnan. In this study, we successfully sequenced the complete genomes of 17 HAstVs, which revealed that the classic HAstV-1 to HAstV-4 strains circulated in Yunnan presenting genetic diversity. HAstV-1

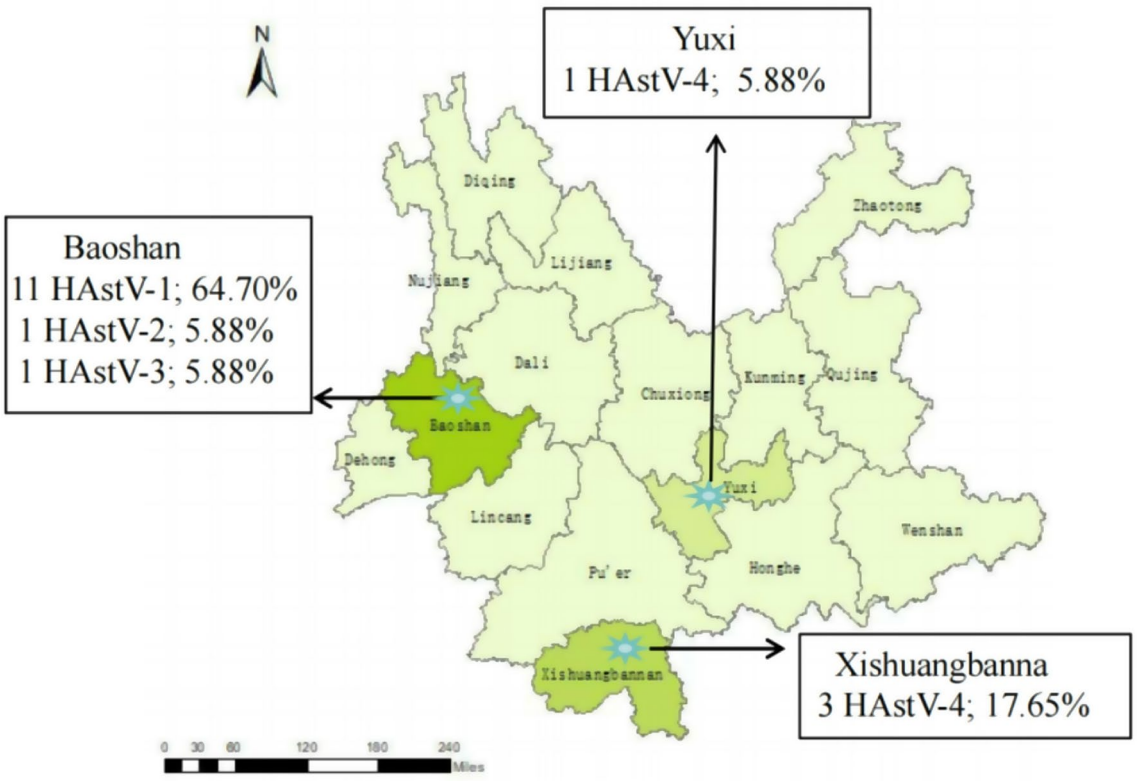

Fig. 4 The genotypes and geographical distribution map of the 17 classic HAstV strains distribution map of classic HAstVs in Yunnan Province(http://www.resdc.cn/data.aspx?DATAID=201)

and HAstV-4 were the predominant genotypes, accounting for 83.33% (15/18) of all the HAstVs.

The distribution of HAstV genotypes differs worldwide, among HAstV genotypes, HAstV-1 is the leading cause of viral gastroenteritis [32–34]. Approximately 90% of the population 9 years and older are reported to have antibodies against HAstV-1 [33]. The dominant strain was consistent with those of previous studies. Although HAstV-1 has 8 subtypes, all 11 HAstV-1 strains belong to subtype 1a. As there is no effective medicine against HAstVs, vaccines are important against HAstVs. In the development of vaccines, HAstV-1 subtype 1a should be considered.

We characterized the prevalence of infection by classic HAstVs among hospitalized children with AGE over a nine-year period (2015–2023), providing an epidemiological overview and molecular characteristics of HAstVs in Yunnan. As only 17 HAstV sequences were obtained, other genotypes circulating in Yunnan were likely not detected. The diversity of HAstV genotypes demonstrated in Yunnan. Different variant and intergenotype recombinant HAstV strains have been reported from Kolkata to cause gastroenteritis in infants, children, and adults [35]. HAstVs should receive increasing attention not only as causative agents of acute gastroenteritis but also as potential pathogens of unexpected diseases. The HAstVs genotypes varied in different regions, HAstV-1 was mainly prevalent in Baoshan, while the predominant strain in Xishuangbanna was the HAstV-4. As the surveillance hospitals were only in 3 regions, and the other regions were unknown in Yunnan. Continuous long-term surveillance covering all the regions is needed to evaluate the effects of HAstVs on people's health in Yunnan.

Conclusion

The genetic diversity of HAstVs was detected. The HAstV-1 to HAstV-4 genotypes were screened in Yunnan, and HAstV-1 was the predominant genotype, followed by HAstV-4. There was no significant difference between male and female patients infected with HAstV. The 24–35 month age group was most affected by HAstV. The highest prevalence of HAstV was in the summer. In the future, thorough and continuous surveillance is needed to explore the epidemiological and evolutionary characteristics of HAstV.

Abbreviations

HAstV	human astrovirus
AGE	acute gastroenteritis
NGS	next-generation sequence
ORF	open reading frame
MLB	Melbourne
VA	Virginia

Acknowledgements
We are very appreciated viral dirahea surveillance staff. for the hard work of Cao Mei and Hu Zhirong from the Yuxi Hongta District Center for Disease Control and Prevention, as well as Dong Yuhui and Yin Xingrui from the Tengchong Center for Disease Control and Prevention.

Author contributions
Yihui Cao wrote the main manuscript text, Wenpeng Gu, Yongming Zhou reviewed the manuscript, Lihua Chen drawed the map. Xiaofang Zhou, Jianping Cun participated the detecting; Lili Jiang analyze the data. Zhichao Wang upload all the sequences to Genbank. All authors reviewed the manuscript.

Funding
Funded by Open Research Fund Program of Yunnan Key Laboratory of Vaccine & Development on Infections Diseases (Grant No. 2020KF001).
Project of the infectious disease spectrum and epidemiology of YNCDC (YNAPM2025–003).

Data availability
The complete genome sequences of HAstV were deposited in the GenBank database with the accession numbers PQ510853–PQ510867 and PQ496890–PQ496891.

Declarations

Ethical approval and consent to participate
The study was approved by the Ethical Review Committee of the Yunnan Center for Disease Control and Prevention. This study was carried out with residual clinical samples. Written informed consent was not required because the patients enrolled in this study were fully anonymized, and verbal informed consent was obtained from the parents or guardians.

Clinical trial
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Yunnan Provincial Key Laboratory of Public Health and Bisosafety & Institute for the Prevention and Control of Acute Infectious Diseases, Yunnan Center for Disease Control and Prevention (Yunnan Academy of Preventive Medicine), Kunming, China

Received: 4 January 2025 / Accepted: 21 February 2025
Published online: 09 March 2025

References

1. Appleton H, Higgins PG, Letter. Viruses and gastroenteritis in infants. Lancet. 1975;1(7919):1297. https://doi.org/10.1016/s0140-6736. (75)92581-7 [published Online First: 1975/06/07].
2. Madeley CR, Cosgrove BP. Letter: 28 Nm particles in faeces in infantile gastroenteritis. Lancet. 1975;2(7932):451–2. https://doi.org/10.1016/s0140-6736(75)90858-2. [published Online First: 1975/09/06].
3. Bosch A, Pintó RM, Guix S. Human astroviruses. Clin Microbiol Rev. 2014;27(4):1048–74. https://doi.org/10.1128/cmr.00013-14. [published Online First: 2014/10/04].
4. Wei H, Khamrin P, Kumthip K, et al. High divergence of human astrovirus genotypes Circulating in pediatric patients hospitalized with acute gastroenteritis in Chiang Mai, Thailand, 2017–2020. Sci Rep. 2021;11(1):23266. https://doi.org/10.1038/s41598-021-02745-7. [published Online First: 2021/12/03].
5. Burke RM, Mattison CP, Marsh Z, et al. Norovirus and other viral causes of medically attended acute gastroenteritis across the age spectrum: results from the medically attended acute gastroenteritis study in the united States. Clin Infect Diseases: Official Publication Infect Dis Soc Am. 2021;73(4):e913–20. https://doi.org/10.1093/cid/ciab033. [published Online First: 2021/08/17].
6. Xavier Mda P, Carvalho Costa FA, Rocha MS, et al. Surveillance of human astrovirus infection in Brazil: the first report of MLB1 astrovirus. PLoS ONE. 2015;10(8):e0135687. https://doi.org/10.1371/journal.pone.0135687. [published Online First: 2015/08/15].
7. Okitsu S, Khamrin P, Hikita T, et al. Molecular epidemiology of classic, MLB, and VA astroviruses in children with acute gastroenteritis, 2014–2021: emergence of MLB3 strain in Japan. Microbiol Spectr. 2023;11(3):e0070023. https://doi.org/10.1128/spectrum.00700-23. [published Online First: 2023/05/04].
8. Wohlgemuth N, Honce R, Schultz-Cherry S. Astrovirus evolution and emergence. Infect Genet Evolution: J Mol Epidemiol Evolutionary Genet Infect Dis. 2019;69:30–7. https://doi.org/10.1016/j.meegid.2019.01.009. [published Online First: 2019/01/15].
9. De Benedictis P, Schultz-Cherry S, Burnham A, et al. Astrovirus infections in humans and animals - molecular biology, genetic diversity, and interspecies transmissions. Infect Genet Evolution: J Mol Epidemiol Evolutionary Genet Infect Dis. 2011;11(7):1529–44. https://doi.org/10.1016/j.meegid.2011.07.024. [published Online First: 2011/08/17].
10. Vu DL, Bosch A, Pintó RM, et al. Epidemiology of classic and novel human astrovirus: gastroenteritis and beyond. Viruses. 2017;9(2). https://doi.org/10.3390/v9020033. [published Online First: 2017/02/22].
11. Gallimore CI, Taylor C, Gennery AR, et al. Use of a heminested reverse transcriptase PCR assay for detection of astrovirus in environmental swabs from an outbreak of gastroenteritis in a pediatric primary immunodeficiency unit. J Clin Microbiol. 2005;43(8):3890–4. https://doi.org/10.1128/jcm.43.8.3890-3894.2005. [published Online First: 2005/08/06].
12. Naccache SN, Peggs KS, Mattes FM, et al. Diagnosis of neuroinvasive astrovirus infection in an immunocompromised adult with encephalitis by unbiased next-generation sequencing. Clin Infect Diseases: Official Publication Infect Dis Soc Am. 2015;60(6):919–23. https://doi.org/10.1093/cid/ciu912. [published Online First: 2015/01/13].
13. Reuter G, Pankovics P, Boros Á. Nonsuppurative (Aseptic) meningoencephalomyelitis associated with neurovirulent astrovirus infections in humans and animals. Clin Microbiol Rev. 2018;31(4). https://doi.org/10.1128/cmr.00040-18. [published Online First: 2018/08/31].
14. Monroe SS, Jiang B, Stine SE, et al. Subgenomic RNA sequence of human astrovirus supports classification of astroviridae as a new family of RNA viruses. J Virol. 1993;67(6):3611–4. https://doi.org/10.1128/jvi.67.6.3611-3614.1993. [published Online First: 1993/06/01].
15. Martella V, Pinto P, Tummolo F, et al. Analysis of the ORF2 of human astroviruses reveals lineage diversification, recombination and rearrangement and provides the basis for a novel subclassification system. Arch Virol. 2014;159(12):3185–96. https://doi.org/10.1007/s00705-014-2153-9. [published Online First: 2014/10/20].
16. Niendorf S, Mas Marques A, Bock CT, et al. Diversity of human astroviruses in Germany 2018 and 2019. Virol J. 2022;19(1):221. https://doi.org/10.1186/s12985-022-01955-3. [published Online First: 2022/12/22].
17. Resque HR, Munford V, Castilho JG, et al. Molecular characterization of astrovirus in stool samples from children in São Paulo, Brazil. Mem Inst Oswaldo Cruz. 2007;102(8):969–74. https://doi.org/10.1590/s0074-02762007000800012. [published Online First: 2008/01/23].
18. Lu L, Zhong H, Xu M, et al. Diversity of classic and novel human astrovirus in outpatient children with acute gastroenteritis in Shanghai, China. Front Microbiol. 2023;14:1265843. https://doi.org/10.3389/fmicb.2023.1265843. [published Online First: 2023/11/29].
19. Ushijima H, Pham NTK, Nishimura S, et al. Human astrovirus infection and outbreaks in Japanese children before and after the emergence of COVID-19 pandemic. J Med Virol. 2024;96(11):e70078. https://doi.org/10.1002/jmv.70078. [published Online First: 2024/11/26].
20. Cao Y, Yang J, Li N, et al. Detection and complete genome sequence analysis of human adenovirus in children with acute diarrhea in Yunnan, China, 2015–2021. Arch Virol. 2024;169(2):34. https://doi.org/10.1007/s00705-023-05950-z.
21. Zhou H, Wang S, von Seidlein L, et al. The epidemiology of Norovirus gastroenteritis in China: disease burden and distribution of genotypes. Front Med. 2020;14(1):1–7. https://doi.org/10.1007/s11684-019-0733-5. [published Online First: 2019/12/12].
22. Zhang P, Zhang J. [Surveillance on other infectious diarrheal diseases in China from 2014 to 2015]. *Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi* 2017;38(4):424– 30. https://doi.org/10.3760/cma.j.issn.0254-6450.2017.04.003 [published Online First: 2017/05/05].

23. Cordey S, Vu DL, Zanella MC, et al. Novel and classical human astroviruses in stool and cerebrospinal fluid: comprehensive screening in a tertiary care hospital, Switzerland. Emerg Microbes Infections. 2017;6(9):e84. https://doi.org/10.1038/emi.2017.71. [published Online First: 2017/09/21].
24. Farahmand M, Khales P, Salavatiha Z, et al. Worldwide prevalence and genotype distribution of human astrovirus in gastroenteritis patients: A systematic review and meta-analysis. Microb Pathog. 2023;181:106209. https://doi.org/10.1016/j.micpath.2023.106209. [published Online First: 2023/06/30].
25. Kim JS, Lee WJ, Lee SK, et al. Molecular epidemiology of human astrovirus in stool samples from patients with acute gastroenteritis in Korea, 2013–2017. Annals Lab Med. 2019;39(4):367–72. https://doi.org/10.3343/alm.2019.39.4.367. [published Online First: 2019/02/28].
26. Mousavi Nasab SD, Zali F, Kaghazian H, et al. Prevalence of astrovirus, adenovirus, and Sapovirus infections among Iranian children with acute gastroenteritis. Gastroenterol Hepatol Bed Bench. 2020;13(Suppl1):S122–27. [published Online First: 2021/02/16].
27. Jarchow-Macdonald AA, Halley S, Chandler D, et al. First report of an astrovirus type 5 gastroenteritis outbreak in a residential elderly care home identified by sequencing. J Clin Virol. 2015;73:115–19. [published Online First: 2015/11/23].
28. Vu DL, Cordey S, Brito F, et al. Novel human astroviruses: novel human diseases? J Clin Virol. 2016;82:56–63. https://doi.org/10.1016/j.jcv.2016.07.004. [published Online First: 2016/07/20].
29. Arowolo KO, Ayolabi CI, Adeleye IA, et al. Molecular epidemiology of astrovirus in children with gastroenteritis in Southwestern Nigeria. Arch Virol. 2020;165(11):2461–69. https://doi.org/10.1007/s00705-020-04741-0. [published Online First: 2020/08/10].
30. Lee SK, Jeon Y, Cho EJ, et al. Surge of human astrovirus type 1 infection in summer 2022 in Korea. Epidemiol Infect. 2025;153:e2. https://doi.org/10.1017/s0950268824000980. [published Online First: 2025/01/06].
31. Tang X, Hu Y, Zhong X, et al. Molecular epidemiology of human adenovirus, astrovirus, and Sapovirus among outpatient children with acute diarrhea in Chongqing, China, 2017–2019. Front Pediatr. 2022;10:826600. https://doi.org/10.3389/fped.2022.826600. [published Online First: 2022/03/22].
32. Toh Y, Harper J, Dryden KA, et al. Crystal structure of the human astrovirus capsid protein. J Virol. 2016;90(20):9008–17. https://doi.org/10.1128/jvi.00694-16. [published Online First: 2016/07/29].
33. Johnson C, Hargest V, Cortez V, et al. Astrovirus Pathogenesis Viruses. 2017;9(1). https://doi.org/10.3390/v9010022. [published Online First: 2017/01/25].
34. Khamrin P, Thongprachum A, Okitsu S, et al. Multiple astrovirus MLB1, MLB2, VA2 clades, and classic human astrovirus in children with acute gastroenteritis in Japan. J Med Virol. 2016;88(2):356–60. https://doi.org/10.1002/jmv.24337. [published Online First: 2015/07/28].
35. Pativada MS, Chatterjee D, Mariyappa NS, et al. Emergence of unique variants and intergenotype recombinants of human astroviruses infecting infants, children and adults in Kolkata, India. Int J Mol Epidemiol Genet. 2011;2(3):228–35. [published Online First: 2011/09/15].

Publisher's note