

ABSTRACT

Background:

Hepatitis A virus (HAV) and hepatitis E virus (HEV) are enterically transmitted viruses that remain important causes of acute viral hepatitis worldwide, particularly in regions with limited sanitation and hygiene structures. In South Africa, limited molecular epidemiological data exist on HAV and HEV infections in pediatric populations. Understanding the circulation of these viruses among children is very important for informing surveillance strategies and public health interventions, including vaccination and sanitation policies. Therefore, the study aimed to determine the prevalence and molecular characteristics of HAV and HEV infections in children under the age of 15 years presenting with acute gastroenteritis in Pretoria, South Africa.

Methods:

A total of 350 archived stool samples collected from children under 15 years of age, presenting with acute gastroenteritis, between 2020 and 2024. These Stool samples were collected from a primary health care facility in Brits and a tertiary academic hospital north of Pretoria. Viral RNA was extracted using the QIAamp viral RNA Mini Kit (Qiagen, Germany). The extracts were initially screened for viral RNA using Real-Time PCR with Bosphore Detection Kits for HAV and HEV (*Anatolia Geneworks, Turkey*). To increase screening efficiency, the extracts were first tested in pools, with individual testing conducted and subsequently tested individually when pools yielded positive results. Positive samples were subjected to confirmatory in-house conventional reverse transcription PCR (RT-PCR) to amplify the VP1/P2B and ORF2/ORF3 regions for HAV and HEV, respectively.

Complementary DNA (cDNA) was synthesized using the Invitrogen SuperScript® III One-Step RT-PCR system with the Platinum Taq High Fidelity DNA Polymerase kit (Thermo-Fisher, USA) using 2870 P forward primer (GACAGATTCTACATTTGGATTGGT) and 3381 N reverse primer (CCATTTCAAGAGTCCACACACT) for HAV as well as HEV361

forward primer (GCR GTG GTT TCT GGG GTG AC) and HEV364 reverse primer (CTG GGM YTG GTC DCG CCA AG) for HEV. Nested PCR was performed using AmpliTaq Gold™ DNA Polymerase (Thermo-Fisher, USA) with the 2896 P forward primer (CTATTCAGATTGCAAATACAAT) and 3289 N reverse primer (AACTTCATTATTTTCATGCTCCT) for HAV, as well as HEV363 primer (GMY TGG TCD CGC CAA GHG GA) and HEV366 primer (GYT GAT TCT CAG CCC TTC GC) for HEV. Amplicons were subjected to Sanger sequencing and phylogenetic genotyping. Statistical analyses were conducted using STATA18, and descriptive statistics were performed to assess prevalence and demographic characteristics.

Results:

Out of the 350 extracts analyzed, the pooled RT-qPCR screening identified 38/350 (10.86%) pools as HAV RNA positive. However, after individual retesting of the positive pools, only 1.14% (n=4) of individual extracts were positive. Only two samples were confirmed to be true positives (0.57%) using RT-PCR and sequencing. Molecular analysis revealed that the confirmed HAV isolates belonged to sub-genotype IB. No HEV RNA was detected in any of the extracts screened. Due to the low number of confirmed positive cases, a comprehensive statistical analysis of risk factors and temporal trends was limited.

Conclusion:

This study detected the low prevalence of HAV RNA among children presenting with acute gastroenteritis in Brits and north of Pretoria, with confirmed cases belonging to HAV sub-genotype IB. No evidence of HEV RNA circulation was detected in the analyzed extracts. The findings contribute to limited molecular data on enterically transmitted hepatitis viruses in South African pediatric populations. However, the low detection rate highlights the need for larger, multisite studies and more sensitive surveillance approaches to better understand the epidemiology of HAV and HEV infections in children.