Seroprevalence and Genotypes of Hepatitis A Virus among Acute Hepatitis Patients and Food Handlers in Kenya

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Abstract

BACKGROUND

Hepatitis A virus (HAV) is a common cause of acute hepatitis worldwide. The epidemiology of HAV is highly dynamic. This necessitates current epidemiological data that can inform actionable recommendations on HAV vaccination and other preventive measures. The study aimed to determine the seroprevalence and genotypes of HAV infections among acute viral hepatitis patients during the January to March 2016 Mombasa outbreak and Kenyan food handlers.

MATERIALS AND METHODS

The study involved 227 healthy food handlers from Bomet County and 43 acute hepatitis patients from Mombasa General Hospital during the 2015–2016 outbreak. This study was approved by the KEMRI Scientific and Ethics Review Unit (SERU No: 2209), and all respondents provided informed written consent. Samples were tested for anti-HAV IgM antibodies to confirm HAV infection. Positive cases underwent nested PCR, sequencing, and genotyping to identify HAV genotypes, providing insights into the genetic characteristics of the circulating strains in these populations.

RESULTS

The mean age of the study subjects was 34.9 years. 16 of 270 (5.93%) samples were positive for anti-HAV IgM antibodies. The HAV seroprevalence was higher among acute hepatitis patients in Mombasa (n=12/43; 27.9%) compared to the food handlers in Bomet (n=4/227; 1.76%). Of the 16 seropositive samples, only four collected from acute viral hepatitis patients were HAV RNA positive. RNA sequencing from these four samples demonstrated the presence of HAV genotype IB (GenBank accession numbers ON832830–832833).

CONCLUSION

The overall HAV seroprevalence rate in our studied populations was 5.93%, with a higher rate among the acute hepatitis patients in Mombasa. HAV genotype IB, associated with water contamination, was observed within the study population.

Keywords: *Acute Hepatitis, HAV, Hepatitis Outbreak, Seroprevalence, Food Handlers, HAV Genotype IB*

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Introduction

The hepatitis A virus (HAV) belongs to the Hepatovirus genus within the Picornaviridae family [1]. Morphologically, the virus is 27 to 28 nm in diameter, with icosahedral symmetry [1]**.** There are two types of infectious HAV particles: naked and quasi-enveloped virions. Quasi-enveloped virions have a lipid membrane and are found in the blood and culture supernatants [2]. Naked virions are quasi-enveloped virions in which the membrane has been removed by the detergent action of bile acids within the biliary canaliculus before they are excreted in the faeces [3].

Despite the lack of antigenic variability, HAV is classified into six genotypes (I–VI), which are in turn divided into sub-types (A and B), of which genotypes I to III can infect humans. Subtype IA is the most common HAV type worldwide [4]. Generally, genetic analysis of HAV strains can provide valuable information regarding the source of the virus in both sporadic and epidemic infections [4].

The transmission of HAV occurs via the fecal-oral route, which includes the consumption of contaminated food or water and person-to-person contact. However, transmission through blood transfusion is noted on rare occasions. Poor hygiene and sanitation practices are reported as the major risk factors for HAV infection, particularly in low and middle-income countries [5].

The World Health Organization (WHO) estimates that approximately 1.5 million people are infected with HAV each year, with an estimated 7134 deaths in 2016. However, the incidence rate of HAV infection is underestimated due to the clinical presentations of this disease since early childhood infection is mainly asymptomatic or present in mild forms [6,7].

Infection with HAV causes an immune response, which is assessed by measurement of specific antibodies: immunoglobulin class M (IgM) anti-HAV antibodies and immunoglobin class G (IgG) anti-HAV antibodies [5,8]. Anti-HAV IgM antibodies are detectable following acute infection, and antibody titers usually decline to zero within 3–6 months [8]. In contrast, anti-HAV IgG antibodies appear within 2–3 months after infection and persist for long, conferring protective immunity against future infections. A majority of hepatitis A seroprevalence studies, therefore, often report anti-HAV IgG and not anti-HAV IgM seroprevalence data [9].

The clinical manifestations of HAV infection range from asymptomatic infection to acute liver failure (ALF) [10]. Common clinical symptoms of HAV infection include jaundice, fever, malaise, anorexia, nausea and abdominal discomfort [5,6,10]. Infection with HAV in early childhood is mainly considered asymptomatic and results in the development of life-long protective immunity [5,6,10]. Globalization and improved sanitary conditions have significantly changed HAV epidemiology [6]. However, an increasing number of individuals are infected at older ages, leading to more severe clinical manifestations and greater disease burden [5,6,10].

Hepatitis A patients typically miss several weeks of work or school, and the costs of supportive medical care can be substantial [5]. Therefore, vaccination against HAV, which is cost-effective, should be prioritized in settings where HAV is a public health concern [11]. Currently, Kenya does not include HAV vaccination in its routine immunization schedule. Despite the recognized burden of HAV in Kenya, there remains a lack of comprehensive data on the prevalence and circulating genotypes of HAV, particularly among specific high-risk groups such as food handlers. To address this need, this study aimed to determine the seroprevalence and molecular characteristics of circulating HAV strains among food handlers and acute hepatitis patients in Kenya.

Materials and Methods

Study area

This study was carried out in Mombasa and Bomet Counties, Kenya. A cohort of 227 healthy food handlers working in the tea factories of Chemogondany (63), Kitumbe (38), Kapset (52), and Rorok (74) in Bomet County were recruited between September and December 2015, and blood samples collected as per the study protocol. Additional samples were collected from 43 patients who had acute severe hepatitis at their admission to Coast General Hospital, Mombasa, between January 2016 and April 2016. Food handlers were selected due to their potential to transmit HAV through food contamination, while acute hepatitis patients provide insights into the circulating strains and disease severity during outbreaks. The comparison between these two groups aimed to assess the difference in HAV seroprevalence between individuals presenting with acute hepatitis and asymptomatic food handlers, providing a broader picture of HAV circulation in different settings. Several factors could confound the relationship between the study groups and HAV infection, including socioeconomic status, hygiene practices, access to clean water and sanitation, and age. While our study did not collect detailed data on all these factors, comparing food handlers and acute hepatitis patients allowed for some inherent control, as both groups likely had similar socioeconomic backgrounds and access to clean water.

Case definition

A case patient was defined by the onset of acute illness with clinical symptoms consistent with hepatitis A, namely fever, malaise, anorexia, nausea, abdominal discomfort, dark urine, and jaundice within the outbreak periods. Socio-demographic (age, gender) and clinical patient data were collected using a standard questionnaire by the clinician attending the patient at the study sites.

Sample collection

The attending clinician drew 5 ml of blood from all enrolled participants. The specimens were refrigerated at 4ºC and shipped to the Center for Virus Research (CVR), KEMRI Nairobi, for pre-analytical processing. The samples were separated by centrifugation at 4000 rpm for 10 minutes, and the serum was aliquoted into two 1.5ml tubes, one for serological assays and the other for molecular assays. The samples were stored at -80º C until use.

Ethics statement

This study was approved by the KEMRI Scientific and Ethics Review Unit (SERU No: 2209). All respondents provided informed written consent. Participant data were anonymized and stored securely to ensure confidentiality.

Virological testing for HAV IgM

The serum samples were thawed for 30 minutes and mixed for 20 seconds. The samples were then tested for HAV-IgM antibodies using an HAV IgM Ab ELISA kit (International Immunodiagnostics Inc, California, USA) according to the manufacturer's instructions. Briefly, 80 µl of assay diluent was dispensed into each well of a microplate containing paramagnetic microparticles coated with hepatitis A virus (human). Subsequently, 20 µl of controls, calibrator, and specimens were added to the appropriate wells, and the contents were gently mixed and then washed five times. 50 µl of acridium-labeled conjugate was added to each well and incubated at 37°C for 1 hour. Following incubation, the wells were washed five times, and 100 µl of working TMB solution was added to each well and incubated in the dark at room temperature for 15 minutes, after which 100 µl of stop solution was added to the reaction mixture. The resulting chemiluminescent reaction was measured as relative light units (RLUs). The presence or absence of IgM anti-HAV in the sample was determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from an Architect anti-HAV IgM calibration. Sero-positive samples were then shipped to the Centre for Microbiology Research Centre, Winnipeg, Canada, for further testing and characterization of HAV isolates.

HAV RNA sequencing and genotyping

HAV RNA was isolated using the NucliSENS easyMAG total nucleic acid automated extraction system (BioMerieux, Quebec, Canada). Nested PCR was then used for HAV amplification using the primers listed in Table 1 in a One-Step RT-PCR reaction. The PCR mixture comprised of 1.75U of Expand High Fidelity polymerase (Roche Diagnostics, Basel, Switzerland), 250 µM dNTPs (Thermo Fisher Scientific, Waltham, Massachusetts, USA), 20 µM of both reverse and forward primers, 1X High Fidelity buffer, and 5 µl of the extracted RNA in a total volume of 50 µl. After the first round of amplification, 5 µl was subjected to a second round of nested amplification reaction utilizing a PCR mixture similar to the initial one but with nested primers (F2 and R2). Gel electrophoresis was subsequently used to identify the positive samples, after which amplicons underwent purification using the Qiagen Gel Purification kit following the manufacturer's recommended protocol. Subsequently, the purified DNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). DNA libraries were then prepared and sequenced using a Big Dye Terminator kit (Applied Biosystems, Foster City, CA, USA) and an automated ABI 3750 XL Genetic Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The resultant sequences were directly assembled and analyzed using Lasergene software suite version 7.1.0 by DNASTAR (Madison, Wisconsin, USA). Alignment and editing of sequences were performed separately using ClustalX version 2.0.1 [12] and Bio-Edit software [13]. The phylogenetic tree was constructed using the maximum-likelihood method and the Tamura-3-parameter with gamma distribution incorporating invariable sites (T92 +G+I) in the MEGA software package (Pennsylvania State University, State College, Pennsylvania, USA) with 2000 replicates. The calculation of the nucleotide identity between the isolates from the index cases and their household contacts was also performed using the MEGA software package (Pennsylvania State University, State College, Pennsylvania, USA).

Statistical analysis

All the socio-demographic and clinical data were entered into Microsoft Access, while laboratory serological test results data were entered into Microsoft Excel. Prevalence was calculated for HAV, and descriptive statistics were generated for the data.

Results

Demographic and clinical characteristics of the participants

This study included two groups of participants: 227 healthy food handlers from Bomet County and 43 acute hepatitis patients from Mombasa General Hospital during the 2015–2016 outbreak.

Table 1:

Primers Used for Amplification of HAV Genome

Primer	Sequence (5'–3')
HAV F1	GACAGATTCTACATTTGGATTGGT
HAV R1	CCATTTCAAGAGTCCACACACT
nested HAV F2	CTATTCAGATTGCAAATTACAAT
nested HAV R2	AACTTCATTATTTCATGCTCCT

Table 2:

Demographic Characteristics of the Study Participants Enrolled in the Study by Gender and Age

Participants	Age range	Mean age	Males	Females
Food handlers	23-48	34.93	153 (67.40%)	74 (32.60%)
Acute hepatitis cases	24-49	34.49	30 (69.76%)	13 (30.24%)

Among the food handlers, 153 (67.40%) were male and 74 (32.60%) were female, with ages ranging from 23 to 48 years (mean = 34.93 years). All food handlers were asymptomatic at the time of enrollment. The acute hepatitis patients included 30 (69.77%) males and 13 (30.23%) females, with ages ranging from 24 to 49 years (mean = 34.49 years). These patients reported clinical symptoms consistent with acute hepatitis, such as fever, nausea, vomiting, jaundice, and dark urine. Table 2.

Serological status

The seroprevalence of anti-HAV IgM antibodies, indicating recent HAV infection, was significantly higher among the acute hepatitis patients (27.91%, 95% CI: 16.1-41.0%) compared to the food handlers (1.76%, 95% CI: 0.7-3.1%) (Table 3).

Detection of HAV-RNA in serum

To assess the presence of active HAV infection, we analyzed HAV RNA levels in serum samples from 12 acute hepatitis patients and 4 food handlers who had tested positive for anti-HAV IgM antibodies. Using nested PCR, HAV RNA was detected in 33% (4/12) of the acute hepatitis patients from Mombasa. However, none of the serum samples from the food handlers in Bomet were positive for HAV RNA (Table 3). The low RNA detection rate among IgM-positive samples could be attributed to several factors, including the timing of sample collection in relation to symptom onset and potential RNA degradation during sample handling and storage, independent of variations in viremia.

Phylogenetic analysis of the HAV isolates

Four of the positive samples were successfully sequenced and were characterized as sub-genotype IB. The strains from different outbreaks showed a nucleotide similarity ranging from 87.4–100%., suggesting that the strains infecting them were the same. The sequences of the four isolates from this study have been deposited in the NCBI (GenBank) and assigned the accession numbers ON832830–832833.

Discussion

Overall, 5.93% of participants across both study populations tested positive for anti-HAV IgM. Among acute hepatitis patients, 27.91% were anti-HAV IgM-positive, while only 1.76% of the apparently healthy food handlers showed positivity. These antibodies are indicative of acute infection, with titers typically declining to undetectable levels within 3–6 months [8]. On average, the incubation period of HAV is 30 days [7,14]. Therefore, our results indicate that the positive cases were most probably infected a few weeks before the sample collection.

The mean age of the study participants was 34.86 years, suggesting that individuals were infected at older ages, confirming previous reports from Kenya that pointed to HAV as an adulthood illness [15,16].

Table 3:
Sero-Prevalence and HAV RNA Positivity Rates among Food Handlers and Acute Hepatitis A Virus Patients

Study site	Positive IgM anti-HAV N (%)	HAV RNA (PCR) N (%)
Chemogondany	0(0)	0(0)
Kitumbe	2(5.26)	0(0)
Kapset	2(3.85)	0(0)
Rorok	0(0)	0(0)
Mombasa acute hepatitis outbreak samples	12(27.91)	4(9.30)
Total	16(5.93)	4(1.48)

Globally, other studies have also confirmed increasing susceptibility in adults [17,18]. Taken together, these findings may indicate a decreasing exposure in childhood [6]. The observed increase in the average age of HAV infection is a concern because it is associated with increased morbidity and mortality [5,19,20].

In this study, 27.91% of acute hepatitis patients were positive for anti-HAV IgM. Similar findings were reported in an indigenous community from North Brazil, where more than 30% of acute HAV cases were identified during an outbreak [21]. These findings, however, are higher than previously reported data from Kenya [15,16,22].

As for food handlers, 1.75 % returned anti-HAV IgM-positive results. This data agrees with a previous study conducted among at-risk groups in Nigeria (1.5%) [23] and the indigenous population in north Brazil (1.7%) [24]. However, other studies recorded lower results than ours. For instance, a lower prevalence of anti-HAV IgM was found in Afro-Brazilian isolated communities (0.6%) [25] and 0.67% in the Kaduna metropolis, Nigeria [26].

A possible explanation for the higher seroprevalence in the Mombasa outbreak data as opposed to the food handlers' study could be selection bias**.** The outbreak data included results from individuals who manifested symptoms of acute hepatitis A status. This could have inflated the seroprevalence among this group in agreement with past WHO reports [14].

To validate the diagnosis, HAV nucleic acid amplification assays might be helpful in the diagnostic process to confirm acute HAV, particularly in individuals who test positive for anti-HAV IgM but have an average or low signal-to-cut off (S/CO) ratio IgM [27]. In our case, anti-HAV IgM-positives had S/CO > 1.00 (Range: 1.027077498 to 7.170868347). For this reason, we performed the detection and sequencing of HAV RNA. As a result, HAV RNA was detected by nested PCR in 25.0% of anti-HAV IgM-positive samples. Notably, all four samples were from acute hepatitis cases and had a S/CO of between 5.294117647 and 6.311858077. Also, variable blood sampling times and the fluctuating nature of viremia during the acute phase may be attributed to the non-detection of HAV RNA among the remaining 12 anti-HAV IgM-positive samples, a fact supported by past studies [28]. Moreover, RNA is inherently unstable and can degrade if not stored or handled correctly, potentially leading to false-negative results. However, this is unlikely to explain the low RNA detection rate among IgM-positive samples in our study, as all samples were carefully managed according to standard operating procedures. Nonetheless, false-positive findings cannot be ruled out since other circumstances can lead to anti-HAV IgM false-positive findings in routine tests. For example, IgM lasts up to 6 months on average, but it can last for a year after the infection has resolved, possibly causing an inaccurate identification of acute hepatitis A [29].

As for the food handlers, 1.75 % returned anti-HAV IgM-positive results, and none had HAV RNA. Our findings conform to previous studies conducted among the indigenous population in north Brazil, which was low, where none of the 1.7% anti-HAV IgM-positive cases had HAV RNA [24]. A phylogenetic analysis of HAV isolates obtained from this study revealed a circulation of the sub-genotype IB in all four isolates. HAV genotype IB, responsible for acute infection in the present study, is associated with water contamination [30]. Mombasa Island relies heavily on piped water supplied by the Mombasa Water Supply and Sanitation Company (MOWASCO), which sources its water from the mainland [31]. However, challenges such as ageing infrastructure, illegal connections, and intermittent supply can compromise water quality and increase the risk of contamination. On the mainland, water sources are more diverse, including piped water, boreholes, wells, and rainwater harvesting. These sources may be more susceptible to contamination due to factors like inadequate sanitation, proximity to open sewers, and flooding, particularly in informal settlements. Our results suggest that the outbreak in Mombasa may have come from a common source, probably contaminated water or food. Future longitudinal molecular surveillance studies should also be done to monitor changes in potential sources, transmission routes and genotypic dynamics of HAV along the Kenyan Coast.

Study Limitations

The findings from this study are subject to a few limitations. First, we did not collect clinical data, including patient socioeconomic status, which limits our ability to describe these variables and their association with HAV seroprevalence. Secondly, most hepatitis A seroprevalence studies report anti-HAV IgG rather than anti-HAV IgM seroprevalence data, as we do here. This is because most studies focus on assessing past exposure to HAV and overall immunity within a population. However, our study aimed to identify recent infections, which is why we focused on anti-HAV IgM, a marker of acute infection. Hence, our findings may not be representative of the general population. While the comparison between food handlers and acute hepatitis patients provided some inherent control for the confounders, we did not collect detailed data on all potential confounding factors, including socioeconomic status, specific hygiene practices, access to clean water and sanitation, and age. These unmeasured confounders might have influenced our findings. Despite these limitations, we are confident that our results provide a valuable baseline for future studies.

Conclusion and Recommendations

Acute viral hepatitis was less prevalent among food handlers than among acute hepatitis patients from the Mombasa outbreak. HAV genotype IB, prevalent in Africa, was also observed within the study population. These findings highlight the need for public health interventions to control HAV infection in Kenya. We recommend incorporating the HAV vaccine into the routine childhood immunization schedule. While HAV infections are usually self-limiting, vaccination ensures early protection, contributes to herd immunity, and helps prevent future outbreaks. Regular monitoring of food and water sources is crucial to ensure safety and prevent contamination with pathogens, including HAV. This includes implementing robust water safety policies, effective treatment methods, and molecular screening techniques to ensure the supply of clean drinking water. Public health authorities should also prioritize community education programs to raise awareness about food- and waterborne diseases and their prevention.

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Authors' contributions

BKL contributed to the design, and data collection and drafted the manuscript. JG and VR analyzed the quantitative data and provided input on interpretation. JB provided input on the design and methods. JD provided input on the design and methods. CO provided input on the design and methods. EKM provided input on the design and methods. AA provided input on the design and methods. SK provided input on interpretation and did a critical review of the manuscript. EMS contributed to the study conception design, interpretation of data and review of the final manuscript. All authors read, revised and approved the final manuscript.

Conflicts of interest. The authors declare that they have no conflicts of interest.

Availability of data statement. All the data have been included in this article. The raw data can be made available on request.

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