Seropositivity of Hepatitis A, B and E Viruses in Two Socio-Economically Distinct Neighbouring Urban Communities in Lusaka, Zambia

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Abstract

Background: Viral hepatitis is prevalent in Africa, but the epidemiological data available are limited. In Zambia, the prevalence of the hepatitis B virus (HBV) is approximately 4-6%, while the hepatitis C virus (HCV) is much less common. The epidemiology of hepatitis A (HAV) and E (HEV) viruses is less defined, and the relationship between HEV and socio-economic status has been scarcely explored. We set out to determine the seropositivity of HAV, HBV and HEV in two adjacent residential areas of urban Lusaka with sharply contrasting housing and wealth.

Methods: A cross-sectional study surveyed adults in a 'high density' (lower SES) and a nearby 'medium density' (higher SES) area. Blood samples were analysed for HAV and HEV IgG antibodies and hepatitis B surface antigen (HBsAg). Demographic and household data were collected; Fisher's exact test assessed statistical significance, while logistic regression compared seropositivity rates.

Results: The study included 59 and 38 participants from low and high SES communities, respectively, of whom 35 (36%) were male, and 62 (64%) were female. Seropositivity to HAV, HBsAg and HEV were 98%, 5% and 26%, respectively. HEV seropositivity was much higher in the low SES community (37%) than in the high SES (8%; P=0.001), but that of HAV and HBV did not differ between the two communities.

Conclusions: The higher seropositivity rate of anti-HEV IgG antibodies in the low SES community compared to the high SES community indicates that poor sanitation and unsatisfactory environmental hygiene conditions are associated with increased risk of hepatitis E exposure.

***Keywords**: Viral hepatitis, Seropositivity, Socio-economic Status (SES), Environmental Hygiene, Public Health*

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Introduction

The five human hepatitis viruses can be transmitted orally (A, E) or parenterally (B, C, D), and are a major global health concern [1]. The hepatitis A virus (HAV) infection is acquired by ingesting contaminated food and drinking water or through person-to-person contact with an infectious individual [2]. High-income countries have very low levels of HAV endemicity compared to low-middle-income countries that have high levels of endemicity, with up to 90% of the total population showing evidence of past infection by the age of 10 years [3]. In 2019, the incidence of HAV was estimated to be 158.94 million globally [4]. According to the Hepatitis Global Report of 2024, an estimated 50 million people are living with the hepatitis C virus (HCV), while 254 million are living with the Hepatitis B virus (HBV) globally [5]. It has been estimated that 20 million hepatitis E virus (HEV) infections occur worldwide, causing approximately 70,000 deaths and 3000 stillbirths annually [6]. Hepatitis E virus (HEV) is increasingly recognised as an important public health problem throughout

Asia and Africa, where many outbreaks occur [7]. HEV outbreaks have also been reported in industrialised countries, where it is frequently a consequence of zoonotic transmission [8].

Previously, HAV and HEV infections have been linked to socio-economic status (SES), environmental hygiene conditions and availability of safe drinking water, but epidemiological data on HEV in Africa are very limited [9,10]. Over 60% of the world's population does not have access to satisfactory sanitation[11,] implying that a considerable proportion of the world's population lives in conditions that promote the survival of these viruses. However, as sanitation standards improve, the proportion of adults susceptible to these infections may decrease, lowering the risk of epidemics [9].

In Zambia, most studies have focused on HBV [12–18], with limited attention given to HAV and HEV[19]. This oversight may be attributed to the high prevalence of the human immunodeficiency Virus, HIV [18] and HIV-HBV co-infections in Zambia, [18,] as these viruses share similar routes of transmission [20,21]. However, neglecting other hepatotropic viruses may have serious health consequences, such as adverse impacts of HEV on pregnancy outcomes. Hence, we carried out a cross-sectional seropositivity survey in two neighbouring communities, namely Misisi compound and Kamwala South, which represent lower and higher SES, respectively. We aimed to investigate the seroprevalence of chronic HBV active infection, HAV and HEV exposure. Additionally, we compared the effects of SES and environmental hygiene on the transmission and infection rates of these viruses. This also allowed us to understand the co-infectivity patterns among these viruses.

Methodology

Study setting

This was a cross-sectional study conducted in two neighbouring communities separated only by a railway line: Misisi compound (very densely populated: low SES) and Kamwala South (less densely populated: high SES). The two communities have visible differences in housing density, infrastructure, and amenities such as roads, water sources and garbage disposal (Table 1). Misisi also has large, uncovered bodies of surface water that were created by quarrying and have subsequently filled up with water, which has become contaminated with solid and liquid wastes.

Recruitment of participants

The two areas were mapped and demarcated into zones, and purposive sampling was conducted to ensure all residents in the chosen contrasting exposure areas were given the opportunity to participate. A known start point was selected, and all households in the two areas were systematically approached to ensure comprehensive coverage and increased chances of a representative sample size. Both areas were consecutively sampled with every household approached by the study team following culturally acceptable norms, with entry only once they were invited in by an adult from the home. Any adults 18 years or older who were home were invited to participate in the study. Households with no one at home or not willing to come to the clinic or provide a blood sample were excluded. All who were willing to take part in the study were invited to St Augustine Clinic, which is situated exactly in between these two communities, close to the railway line. At the clinic, a trained nurse explained the study's objectives and obtained written consent from the participants. She then administered a guided questionnaire and collected 5ml of blood. The questionnaire included pre-tested questions that gathered information on SES factors such as education level, occupation, car ownership, and marital status, as well as sanitation and hygiene status, including water sources, type of toilet, number of people in the household, and garbage disposal.

Testing for the Hepatitis B virus surface antigen (HBsAg) was conducted immediately by the study nurse at recruitment using the Alere Determine™ HBsAg Rapid test kit (Abbot, Illinois, USA) according to the manufacturer's instructions. Test results were communicated to the participants following pre- and post-counselling sessions. The collected blood was sent to the laboratory, where it was centrifuged to separate the plasma, which was then stored at -80°C until further serological testing.

Laboratory-based serological testing

Plasma samples were used to test for IgG anti-HAV and anti-HEV antibodies using the enzyme-linked immunosorbent Assay (ELISA) (Fortress Diagnostics, Antrim, United Kingdom), following the manufacturer's instructions. The IgG anti-HEV ELISA is a qualitative indirect ELISA that uses an anti-human globulin conjugate to detect the IgG antibodies present in the sample, while the IgG anti-HAV ELISA is a qualitative solid-phase competitive ELISA that detects IgG anti-HAV present in the sample. Using cut-offs set accordingly, the positivity and negativity of IgG anti-HAV and HEV in all study samples were determined.

Statistical analysis

Participant characteristics, such as sex, age, level of education, occupation, number of people per household, type of garbage disposal, water source, and type of toilet used, were summarised according to the area of residence (Table 1). The distribution of age (the only continuous variable) was not normal; therefore, the summary statistics are presented as medians and interquartile ranges. Seropositivity for HAV, HBV and HEV was analysed as proportions, and statistical significance for categorical variables was tested using Fisher's exact test. Logistic regression was used for multivariable analysis with sex and age as the only covariates, as all other variables are associated with SES. All analyses were performed using STATA software, version 17 (Stata Corporation, College Station, TX, USA). Sample size calculations (using the power twoprop command in Stata 17.0) indicate that the power available to detect a 29% difference in HEV seropositivity between the two groups was 93%.

Ethical considerations

All participant data was de-identified and accessible to study staff only. Recruitment was performed according to the Helsinki Declaration. Approval for this study was obtained from the University of Zambia Biomedical Research Ethics Committee on the 10th of May 2018 under the clearance number 003-03-18.

Results

Between July and October 2018, 777 households were approached (149 from Kamwala South and 627 from Misisi), and adults from these households were invited to St. Augustine Clinic. Of the 149 households that were approached in Kamwala South, 40 (27%) did not respond, 40 (27%) stated that they did not wish to be interviewed, and 31 (21%) were not willing to provide a blood sample; hence, only 38 participants from Kamwala South were recruited. Of the 627 households approached in Misisi compound, 200 (32%) did not respond, 149 (24%) declined to be interviewed, and 219 (35%) households did not provide a blood sample. This left 59 participants who completed the study (see Figure 1). There were more females than males in Misisi compound ($P = 0.009$), while the number of males and females in Kamwala South was comparable (shown in Table 1).

The dominant age group of participants was between 18 and 49 years old, with a median age of 33 years ($P = 0.99$). In Kamwala South, 50% had reached tertiary education, while the highest level of education in Misisi Compound was secondary education, which was attained by 34% of the participants. Most of our participants were self-employed (55% from Kamwala South and 42% from Misisi; $P = 0.50$) with 6 (IQR; 4-7) and 6 (IQR; 5-9) people per household living in Misisi compound and Kamwala South, respectively. In Kamwala South, 97% of participants were registered with garbage collection services, while all participants in Misisi compound disposed of their garbage in the open ($P < 0.0001$). Almost all study participants had access to clean water, with 95% of those from Kamwala South using flushable toilets and 100% from Misisi Compound using pit latrines ($P < 0.0001$) (Table 1).

Anti-HAV IgG and HBsAg seropositivity

Across sites, 95 (98%) participants tested positive for anti-HAV IgG antibodies (i.e. 59/59 (100%) from Misisi and 36/ 38 (95%) from Kamwala South). Only 5 participants tested positive from both sites (5.2%) (Shown in Table 2).

Anti-HEV IgG seropositivity

From both communities, 25 (25.8%) adults were positive for anti-HEV IgG, of which 22 were from Misisi Compound and 3 from Kamwala South (OR 6.9, 95% CI 1.8, 38.6; *P=0.002*; Table 2). HEV seropositivity was detected in 12 of 62 women and 13 of 35 men (OR for female sex 0.41, 95%CI 0.16,1.03; *P=0.06*).

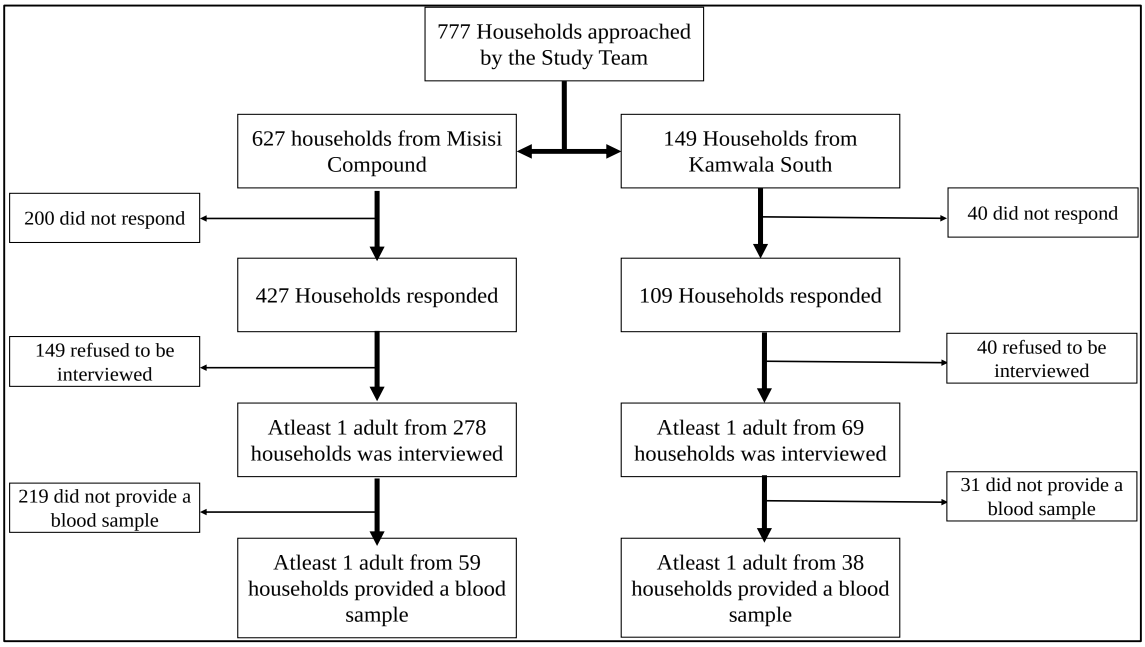

Figure 1
Flowchart Summarising Study Participant Recruitment

Table 1
Demographic and SES characteristics of all Study Participants

	Location		
Characteristics	**Kamwala South (n=38)**	**Misisi (n=59)**	**P-value**
Sex M	20(53)	15(25)	0.009*
Age (median (IQR), years)	33(24,45)	33(24,46)	0.99
Level of Education			<0.0001*
None	0(0)	4(7)	
Primary	4(11)	35(59)	
Secondary	15(39)	20(34)	
Tertiary	19(50)	0 (0)	
Occupation			0.50
Unemployed	12(32)	23(39)	
Self-employed	21(55)	25(42)	
Formally Employed	5(13)	11(19)	
Number of people per household			0.16
Median (IQR)	6 (4-7)	6 (5-9)	
Type of garbage disposal			<0.0001*
Open	0	59 (100)	
Garbage collectors	37 (97)	0 (0)	
Burial	1 (3)	0 (0)	
Water Source			0.02*
Tap Water	34(89)	59(100)	
Borehole	4(11)	0(0)	
Toilet Used			<0.0001*
Flushable	36(95)	1(2)	
Pit-latrine	2(5)	58(98)	

*Denotes all significant differences

Using logistic regression, the odds of anti-HEV IgG antibodies were greatly increased in Misisi compared to Kamwala South (adjusted odds ratio 15.3; 95% confidence interval, 3.4- 68.5; *P<0.0001*), and being female was significantly protective against HEV (adjusted OR 0.16, 95%CI 0.05, 0.52; *P=0.003*, hence, being male increased the odds of acquiring HEV (by 6.34 times) and living in Misisi increased the odds of having HEV by 15.3 times (*P<0.001*).

Hepatitis virus co-infections

Summarising the number of hepatitis co-infections in the participants for each site, 1 (1.7%) participant from Misisi compound was positive for all 3 hepatitis viruses, while 24 (40.7%) were positive for two viruses (21 for HAV/HEV and 3 for HBsAg/HAV) (shown in Figure 1b). From Kamwala South, only 3 participants were co-infected with HAV/HEV (8%) and 1 participant with HBsAg/HEV (3%) (shown in Figure 2a).

Discussion

We conducted a seroprevalence study of three important hepatitis viruses in two adjacent communities, separated by a railway line and significant differences in SES. Although the seropositivity of HAV and HBV was similar in both communities, there was a significant difference in the prevalence of HEV. The high prevalence rate of anti-HAV antibodies observed in both communities is entirely consistent with prevalence in many low-income countries [19] and indicates high levels of exposure to HAV across the SES strata. In previous studies, we have demonstrated that consuming water in Misisi may present a higher risk of infection due to coliform bacterial contamination [22,23]. The source of HAV may be difficult to isolate in these two areas, but a reasonable prediction may be through the ingestion of contaminated food and drink, either at home or as people are out for work or business.

Table 2

Seropositivity of HBsAg, Anti-HEV and Anti-HAV IgG in Study Participants by SES.

		Misisi (low SES)	Kamwala South (higher SES)	OR (95%CI)	P value
HBsAg	Pos	4 (6.8)	1 (2.6)	2.7 (0.25,136)	0.65
	Neg	55	37		
HEV	Pos	22 (37.3)	3 (7.9)	6.9 (1.8,39)	0.002*
	Neg	37	35		
HAV	Pos	59 (100)	36 (95)	-	0.15
	Neg	0	2		

OR cannot be calculated for HAV as one cell has zero counts.
Positive; n (%)

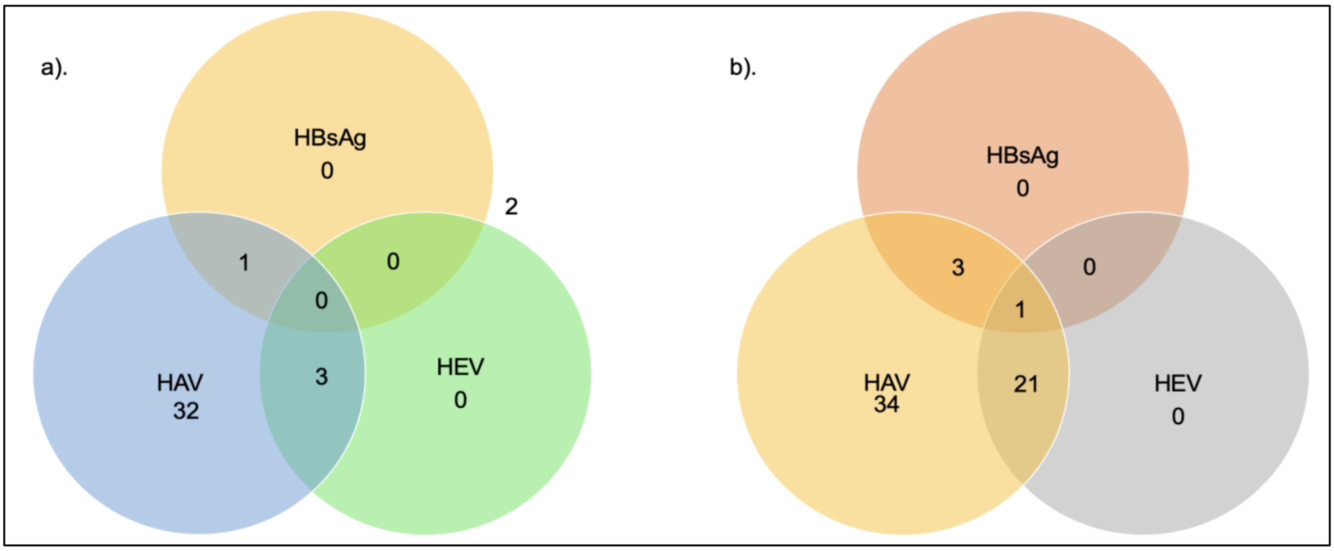

Figure 2

Venn Diagrams Summarising the Number of Hepatitis Virus Co-Infections in the Study Participants; a) Kamwala South (high SES) and b) Misisi Compound (low SES)

Food as a route of exposure is supported by a review that reported several HAV outbreaks in the United States between 1968 and 2003 that all started due to ingestion of contaminated food at the point of sale or service [24]. The consequences of such a high HAV exposure for the health of the residents of these areas are unclear. Based on this data, we did not find any evidence of reduced HAV exposure in residential areas of Lusaka with improved sanitation. However, we recommend further work beyond the two communities sampled.

We found a HBsAg seropositivity of 5%, which was not statistically different between the two communities. These findings are consistent with the 5.4% rate reported in 2016 in Zambian adults aged between 15 and 59 years [18]. However, a study of 4961 Zambian adults, published in 2020, reported a prevalence of 3.7%, lower than the national prevalence of [14] or the current study. The effective prevention of HBV infection can be achieved through vaccination, which is a public health priority in Zambia. Since 2005, vaccination against HBV has been included in the childhood vaccination program, though it does not cover infants in their first six weeks of life [25].

The seroprevalence of HEV in Misisi was found to be high at 37%. This is comparable to a similar study conducted in Misisi in 2014, which also demonstrated an association with HIV infection [19]. Our findings regarding HEV prevalence align with those of a Korean study conducted in 2007 [26]. That study identified male gender, low levels of education, and living in rural areas as factors associated with higher HEV seroprevalence rates. The observed male predominance in HEV infection remains unexplained; potential reasons could include greater exposure outside the home or inherent differences in host immunity. The significant differences in HEV exposure between the two communities we surveyed suggest that HEV infection may serve as an indicator of varying environmental exposure to enteropathogens. This could imply that HEV is less infectious than HAV, which was detected in nearly all adults sampled.

Although the consequences of co-infections with HEV and other viral hepatitis infections remain unclear, HEV alone has been associated with several adverse outcomes. These include spontaneous abortions, premature rupture of membranes, preterm births, stillbirths, and neonatal complications [27,28]. Therefore, further research is necessary to understand the impact of HEV on negative maternal and neonatal outcomes in Zambia. A study focusing on co-infection with HAV and HEV revealed significantly higher levels of transaminases, with five participants developing acute liver failure compared to just one participant with HAV mono-infection [29]. Additionally, a 2019 review indicated that HEV co-infection with HBV might increase the risk of cirrhosis, decompensated liver disease, and mortality [30]. Another review noted that patients infected with HEV could develop extra-hepatic disorders, including blood, neurological, and renal disorders [31]. These findings suggest that HEV could have serious implications for health and clinical outcomes. Understanding the transmission dynamics and prevalence of HEV in the Zambian population is critical.

Limitations of the study

This study is limited firstly by its exploratory nature and the relatively small proportion of respondents who consented to testing, making the representativeness and generalizability of the findings difficult. Our low recruitment rate likely resulted from study staff approaching households when most adults were absent, possibly due to work. Refusals may stem from misunderstanding, lack of interest, fear of needles, or reluctance to visit the clinic. Consequently, we might have enrolled participants with similar characteristics due to the limited and convenient method of sampling. Secondly, the absence of anti-HAV and anti-HEV IgM data restricted the assessment of active or recent infections. Thirdly, no liver function tests were performed, thereby limiting insights into the clinical status or progression of viral hepatitis among participants. Fourthly, the genotype of circulating HEV strains was not determined, which could be important given the potential genotype-specific differences in clinical outcomes. Finally, the lack of information on participants' travel histories constrained the evaluation of possible transmission routes and sources of infection.

Conclusions

The prevalence of HBsAg in Zambia has remained relatively stable in recent years. While national seroprevalence data for HAV and HEV are lacking, these findings indicate a likely high burden of anti-HAV and anti-HEV IgG in communities comparable to those studied. Given the elevated seroprevalence of HEV, there is an urgent need to investigate its potential impact on pregnancy outcomes in sub-Saharan Africa.

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Author contributions

- PK, ES and MM designed the study
- AK and JM designed the data collection and implemented it
- TNP, MM and PK analysed the data and wrote the draft manuscript, which was approved by all authors.

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