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Serologic evidence for hepatitis E virus infection among patients with undifferentiated acute febrile illness in Kibera, Kenya

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Abstract

Background—Hepatitis E (HEV) is an emerging cause of viral hepatitis mainly transmitted through the fecal-oral route. Residents of the Kibera slum of Nairobi, Kenya are at risk for fecal-orally transmitted infections.

Objective—To quantify the incidence and prevalence of HEV infection among acute febrile illness (AFI) cases using a population-based infectious disease surveillance network.

Study Design—Cross-sectional serum samples from AFI case-patients between 2009 and 2012 were matched to the age and gender distribution of the Kibera population and tested by IgM and IgG enzyme immunoassays (EIA) and nucleic acid testing (NAT). Serum from healthy residents was also tested by EIA.

Results—Of the 482 AFI serum samples tested, 124 (25.7%) and 182 (37.8%) were IgM and IgG reactive, respectively. On multivariate analysis, IgM reactivity was associated with HIV (RR 1.66, 95%CI 1.07, 2.60; p=0.024) while IgG reactivity was associated with increasing age (p<0.001) and HIV (RR 1.93, 95%CI 1.52, 2.46; p<0.001). AFI case-patients were more likely to be IgM (p=0.002) and IgG (p<0.001) reactive compared to healthy residents. The seroincidence by HEV-specific IgM was 84.0 per 1,000 person years, however, all 482 samples were negative by NAT.

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Disclaimer

The conclusions in this report are those of the authors and do not necessarily represent the official position of the CDC.

Competing Interests

The authors have no financial or personal conflicts of interest to report

Ethical Approval

Ethical Review Board, KEMRI (#932). Institutional Review Board, CDC (#4566).

Conclusions—Serologic evidence for HEV in Kibera suggests a high burden of infection, but NAT did not confirm HEV viremia. Additional testing is needed to determine whether EIAs are susceptible to false positivity in undifferentiated AFI populations before their widespread use.

Background

Globally, there are an estimated 20 million hepatitis E virus (HEV) infections, 3 million cases of symptomatic HEV, and 70,000 HEV-related deaths each year [1]. In East Africa, large outbreaks have been documented, most recently in Uganda, South Sudan, and Kenya [2–4]. While data on HEV endemicity in this region is scarce, an acute jaundice surveillance system in Uganda found that 42% of cases were due to HEV infection [5] suggesting that HEV may be an under-recognized cause of viral hepatitis in Africa.

Currently, HEV testing consists of identifying HEV-specific host antibodies with enzyme immunoassays (EIAs) or HEV RNA through nucleic acid testing (NAT) methods [6]. Both IgM and IgG are detectable at the time of symptom onset. IgM wanes after several weeks while IgG remains detectable for years. HEV RNA is detectable by NAT in serum or stool with peak RNA levels occurring during late incubation and lasting approximately 2–3 weeks after symptom onset [6]. NAT validation of IgM EIAs demonstrate a sensitivity and specificity that vary from 72–98% and 78–95%, respectively [7].

The clinical presentation of HEV infection varies from asymptomatic illness to fulminant hepatitis. The majority of HEV infections are mild with nonspecific symptoms resembling a non-specific febrile illness and patients may not necessarily seek health care [6]. Jaundice occurs in approximately 40% of cases.

Objective

Determine the incidence and prevalence of HEV infection in a population-based sample of undifferentiated acute febrile illness (AFI) cases in Kibera, Kenya.

Study Design

Setting

Kibera, located within the city of Nairobi, is the largest urban slum in Africa. The US Centers for Disease Control (CDC) and the Kenya Medical Research Institute (KEMRI) have conducted population-based infectious disease surveillance (PBIDS) in Kibera since 2005 in an area with an estimated population of 25,000–29,000 individuals in 0.37km^2 area. Due to the high population density and lack of sufficient clean water and proper sanitation, fecal-orally transmitted diseases account for a large proportion of the infectious disease burden in Kibera [8].

Participants who have resided in Kibera for at least 4 months are eligible for enrollment in PBIDS and are visited biweekly for screening. The undifferentiated AFI cohort includes participants who either present to a clinic or are detected by household visits and have an axillary temperature ≥38.0°C without an obvious source [8]. Each AFI case-patient submits blood, stool, and nasopharyngeal samples for clinical diagnosis and research purposes within

7 days of onset of illness. The samples are stored at −80°C in freezers with backup generators maintained by the CDC.

Specimen Analysis

Serum samples collected between 2009 and 2012 from randomly selected AFI case-patients matched by the age and gender distribution of Kibera were tested by EIA and NAT. Each sample was tested by EIA in duplicate for IgM and IgG-specific HEV antibodies (DS-EIA-ANTI-HEV-G, DS-EIA-ANTI-HEV-M. IgM sensitivity (98.0%) and specificity (95.2%) [7]). Results were averaged to generate a signal-to-noise ratio with ≥1.0 designated as positive per the manufacturer's recommended cutoff. Each serum sample underwent HEV NAT by both reverse transcription and real-time polymerase chain reaction protocols developed by CDC [9]. Univariate and multivariate log-binomial regression analysis of demographic and EIA data were computed with Stata 13. The total number of AFI cases during the study period were used to calculate incidence.

Specimens from healthy residents presenting to the clinic with non-infectious complaints were selected by matching the age and gender distribution of Kibera and were tested with HEV EIA. Student's T-test was used to compare the rate of IgM and IgG-reactive subjects in the AFI and healthy groups.

Results

Of the 482 AFI case-patients, 51.2% were female, the average age was 20.4 years, 9 individuals (1.9%) had jaundice, and HIV status was unknown in 293 (60.1%), positive in 34 (7.1%), and negative in 155 (32.2%). HEV EIA identified 124 (25.7%) IgM reactive and 182 (37.8%) IgG reactive case-patients. Among HEV EIA reactive samples, the majority were weakly reactive for both IgM (median signal-to-noise ratio: 1.4, range: 1.0–5.9) and IgG (median signal-to-noise ratio 1.38, range: 1.0–8.3). All samples were negative for HEV RNA by both NAT methods.

On univariate analysis, IgM reactivity was associated with female gender (p=0.049) while IgG reactivity was associated with older age (p≤0.001) (Table 1). IgM (p=0.003) and IgG (p≤0.001) reactivity were both associated with HIV infection. There was no statistically significant association between jaundice and IgM (p=0.598) or IgG (p=0.266) reactivity. On multivariate log-binomial regression, only HIV was associated with IgM reactivity (RR 1.66, 95%CI 1.07, 2.60; p=0.024) (Table 2). IgG reactivity was associated with increasing age (p<0.001 for trend) and HIV positivity (RR 1.93, 95%CI 1.52, 2.46; p<0.001).

During the study period, there were 25,353 AFI cases accounting for 77,555 person years. The HEV IgM seroincidence was estimated to be 84.0 per 1,000 person years while HEV IgG prevalence was 37.8%.

The 57 healthy residents tested for comparison had a similar age (p=0.771), gender (p=0.342), and HIV status (p=0.661) distribution as the AFI sample. Four samples (7.0%) were IgM reactive while 1 sample (1.8%) was IgG reactive. AFI case-patients were more

likely to be IgM and IgG reactive than healthy subjects by student's T-test (p=0.002 and p<0.001, respectively).

Discussion

The seroincidence of HEV infection in Kibera among persons with undifferentiated AFI was high based IgM EIA reactivity. The HEV seroincidence is similar to rates observed in a Bangladeshi cohort [10]. However, reconciling the high rate of IgM positivity with the absence of RNA by NAT is challenging as the former may represent false positives while the latter may represent false negatives. While the HEV EIA used has favorable performance characteristics [7], its validity in undifferentiated AFI is not known. Similarly, while HEV NAT is a highly sensitive test with its low limit of detection [11], the absence of HEV RNA in itself does not exclude true HEV-specific IgM antibodies.

The absence of HEV RNA in the setting of IgM positivity has been previously noted in both a Ugandan acute jaundice syndrome cohort and a Cambodian AFI cohort where only 58% and 1.0% of IgM positive cases had detectable RNA, respectively [2, 12]. The investigators hypothesized that alternative infectious agents may cause cross-reactivity of HEV EIAs, resulting in false positives. Other research has demonstrated EIA cross reactivity between EBV and CMV [13–14], hepatitis A [15], and HIV [16–17], suggesting a 'molecular mimicry' phenomenon. Our findings of a significant association between HIV and HEV IgM positivity, lack of HEV RNA, and lower rates of HEV EIA positivity among healthy control residents may be suggestive of such a process. The high rate of IgG positivity also may suggest a similar phenomenon. Convalescent serum samples were not available for confirmatory testing of this hypothesis.

Previous studies in the East African region demonstrate that respiratory viruses account for the majority of febrile illness [18–19], malaria was an infrequent cause of fever in Kenya (≤5%) [19–20], and viral hepatitis was rare [18]. Unfortunately, data on these concomitant infections were not available for our analysis.

To further elucidate the validity of the HEV IgM EIA, we propose testing of AFI incident and convalescent sera to determine if there is a change in titers of IgM after initial positivity and persistence of IgG positivity expected in true HEV infections. HEV immunoblot may also be used to validate the IgM EIA given its high specificity [21]. In addition to further testing for concomitant infections to assess for molecular mimicry, plaque reduction neutralization testing can be used to identify antibodies against both HEV and other infections.

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Highlights

- Hepatitis E (HEV) diagnosis consists of indirect testing with enzyme immunoassays and direct testing with nucleic acid testing (NAT)
- Serologic evidence suggests a high level of HEV infection among persons with undifferentiated AFI in Kibera
- NAT did not corroborate a high level of HEV infection among our AFI sample population
- Serologic HEV testing in a population with undifferentiated AFI may yield false positives due to cross-reactivity from other infectious agents.

Table 1

Demographic by Hepatitis E Enzyme Immunoassay Result Comparing IgM and IgG Reactive Subjects

Characteristics	IgM Reactive n= 124	IgM Non-Reactive n= 358	p-value¶	IgG Reactive n= 182	IgG Non-Reactive n= 300	p-value¶
Female (%)	73 (58.9)	174 (48.6)	0.049	94 (51.6)	153 (51.0)	0.890
Age (%)			0.074			<0.001
<15 years	43 (34.7)	173 (48.3)		46 (25.3)	170 (56.7)	
15–30 years	40 (32.3)	91 (25.4)		57 (31.3)	74 (24.7)	
30–45 years	29 (23.4)	67 (18.7)		55 (30.2)	41 (13.7)	
≥45 years	12 (9.7)	27 (7.5)		24 (13.1)	15 (5.0)	
HIV (%)			0.003			<0.001
Negative	45 (36.3)	110 (30.7)		58 (31.9)	97 (32.3)	
Positive	16 (12.9)	18 (5.0)		27 (14.8)	7 (2.3)	
Unknown	63 (50.8)	230 (64.2)		97 (53.3)	196 (65.3)	
Jaundice (%)	3 (2.5)	6 (1.7)	0.598	5 (2.8)	4 (1.3)	0.266

¶P-values derived from chi-squared test (Female, HIV, Jaundice) and Student's T-test (Age)

Table 2

Univariate and Multivariate Log-Binomial Regression of Demographics against IgM and IgG Reactive Subjects

Characteristics	IgM Reactive (n=482)		IgG Reactive (n=482)	
	Bivariate Analysis RR (95% CI; p-value)	**Multivariate Analysis RR (95% CI; p-value)**	**Bivariate Analysis RR (95% CI; p-value)**	**Multivariate Analysis RR (95% CI; p-value)**
Female	1.36 (1.00, 1.86; p=0.051)	1.36 (0.99, 1.86; p=0.058)	1.02 (0.81, 1.28; p=0.890)	0.95 (0.79, 1.15; p=0.612)
Age				
<15 years	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
15–30 years	1.53 (1.06, 2.22; p=0.024)	1.29 (0.86, 1.95; p=0.220)	2.04 (1.48, 2.82; p<0.001)	2.17 (1.55, 3.03; p<0.001)
30–45 years	1.52 (1.01, 2.28; p=0.044)	1.13 (0.71, 1.81; p=0.597)	2.69 (1.97, 3.66; p<0.001)	2.62 (1.90, 3.61; p<0.001)
≥45 years	1.55 (0.90, 2.66; p=0.115)	1.48 (0.85, 2.59; p=0.166)	2.89 (2.02, 4.13; p<0.001)	3.06 (2.21, 4.25; p<0.001)
HIV (%)				
Negative	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
Positive	1.62 (1.05, 2.50; p=0.029)	1.66 (1.07, 2.60; p=0.024)	2.12 (1.63, 2.77; p<0.001)	1.93 (1.52, 2.46; p<0.001)
Unknown	0.74 (0.53, 1.03; p=0.074)	0.81 (0.57, 1.17; p=0.264)	0.88 (0.68, 1.15; p=0.357)	1.28 (0.99, 1.65; p=0.058)