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Declining hepatitis E virus antibody prevalence in Phnom Penh, Cambodia during 1996–2017

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Abstract

Hepatitis E virus (HEV) infection is endemic in Cambodia. However, little relevant data were available and there is no clue if HEV is an emerging or decreasing pathogen in that setting. The aim of our study was to describe temporal trends of anti-HEV IgG and IgM prevalences during the last two decades (1996–2017) in the context of population growth and urbanisation in Cambodia. A total of 2004 human plasma samples collected between 1996 and 2017 were tested for anti-HEV IgG and IgM using the commercial Wantai anti-HEV assays. Overall, the prevalences of anti-HEV IgG and IgM were 41.1% and 2.7%, respectively. Analysis by calendar period showed a decreasing trend of anti-HEV IgG prevalence over the last 21 years. After age- and gender-standardisation, the anti-HEV IgG prevalence rates decreased from 61.3% during the 1996–2000 period to 32.3% during the 2016–2017 period, but no trends were observed for anti-HEV IgM rates, which fluctuated around the overall one. In conclusion, our results suggest that HEV is not an emerging pathogen, but rather seems to circulate less in Cambodia, in particular, in Phnom Penh, since the prevalence of anti-HEV IgG has been significantly decreased during the past two decades.

Introduction

Hepatitis E virus (HEV), a positive-sense single-stranded non-enveloped virus, is the causative agent of hepatitis E. It belongs to the genus Orthohepevirus within the family of Hepeviridea and was divided into seven genotypes, numbered 1–7, differing in geographical distribution, modes of transmission and hosts [1, 2]. Four genotypes (genotype 1–4) have been known to be responsible for infection in human. Genotype 1 and 2 are restricted to human and mainly transmitted through faecal-oral route due to contaminated drinking water, whereas genotype 3 and 4 are a zoonotic virus but can infect human as an accidental host through consumption of contaminated food [3, 4]. In healthy individuals, HEV infection is self-limiting and usually resolves within 2–6 weeks. However, development to chronic infection has been reported in some populations, notably in immunocompromised patients such as organ-transplant recipients and human immunodeficiency virus (HIV)-infected patients [5, 6]. Furthermore, a serious complication, known as acute fulminant hepatitis has been observed among at-risk populations including the elderly, pregnant women, patients with existing liver disease and immunocompromised individuals [7].

HEV is present worldwide and infects about one-third of the world population [8]. Epidemiology of HEV can be divided into four distinct geographical zones: (1) hyperendemic zone, where HEV causes over 50% of endemic acute viral hepatitis, counting many countries in Asia, Africa and South America; (2) endemic zone, where HEV causes between 25 and 50% of endemic acute viral hepatitis, counting some countries in the Middle East, Southeast Asia (Singapore) and South America; (3) distinctive zone concerning only Egypt where the HEV epidemiology is different from other parts of the world as HEV infects predominantly children; and (4) sporadic zone corresponding to the remaining part of the world includes Europe and North America [8, 9].

In Cambodia, a South-Eastern Asia country, very little information about HEV infection was available. Three recent cross-sectional surveys indicated an endemic characteristic of HEV epidemiology in the country [10–12]. The first investigation performed from 2010 to 2014 on 868 healthy individuals (median age: 29 years) living in rural area of Siem Reap (a province in North-Western Cambodia), estimated the rate of anti-HEV IgG at 18.4% [12]. In previous studies, our team confirmed the endemicity of HEV in the different population including subjects with or without fever, subjects with or without liver enzyme elevation, severely immunocompromised HIV-1 infected patients and blood donors [10, 11]. All of these studies reported

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HEV prevalence within a limited calendar period of time and could not give a hint of a trend of HEV infection in Cambodia.

To describe the temporal trend of HEV infection in the context of population growth and urbanisation in Cambodia, we determined retrospectively the anti-HEV IgG and IgM prevalences during a period of 21 years (1996–2017) using the same diagnostic ELISA kits.

Methods

Study design and sample collection

This is an observational epidemiological study performed retrospectively on individually unidentifiable leftover and frozen (−80 °C) plasma samples that were collected from adult individuals (⩾18 years) coming to the Medical Laboratory of Institut Pasteur du Cambodge for their routine medical check-up between 1996 and 2017 and that would have been otherwise discarded. All samples have been kept frozen until use in the present study. Gender, age and town or province of residence were systematically recorded. The present study was approved by the Cambodian National Ethics Committee for Human Research (No. 173NECHR).

HEV testing

All specimens were screened for anti-HEV IgG using the commercial Wantai HEV IgG ELISA kit (Beijing Wantai Biological Pharmacy Enterprise, Beijing, China). To investigate recent HEV infection, all positive anti-HEV IgG specimens were further tested for anti-HEV IgM using the commercial Wantai HEV IgM ELISA kit. All serological assays were performed according to the manufacturer's instructions. All specimens positive for anti-HEV IgM were tested for the presence of HEV RNA using a quantitative in-house HEV RNA RT-PCR [13]. In brief, HEV RNA was extracted from 140 µl of plasma using manual nucleic acid extraction (QIAamp viral RNA mini kit, Qiagen). The RT-polymerase chain reaction (PCR) targeted a highly conserved domain within the ORF3 region of the HEV genome. The sensitivity threshold of the RT-PCR was 125 IU/ml, as determined by the First (2011) WHO standard for HEV RNA nucleic acid test-based assays (#6329/10, Paul Ehrlich Institute, Langen, Germany).

Statistical analysis

Patients' characteristic were compared by calendar periods (1996–2000, 2001–2005, 2006–2010, 2011–2015 and 2016–2017) using a χ^2 test for categorical variables and a Kruskall–Wallis test for continuous variables.

Anti-HEV IgG prevalence and its 95% confidence interval (CI) were estimated for each calendar period. We defined that a total of 170 of patients per time period would allow to detect a 15% difference of the anti-HEV IgG prevalence, as long as the highest prevalence does not exceed 50% with a significance level of 5% and a power 80%. However, to improve statistical power, we considered all samples from each calendar period.

To better investigate the calendar effect, a gender and age-standardisation of the anti-HEV IgG prevalence was conducted using the 2014 Cambodian census [14]. To quantify the effect of the calendar period, a logistic regression analysis was conducted by considering also gender, age group (18–29, 30–39, 40–49, ⩾50 years) and place of residence (Phnom Penh *vs.* other) for adjustment and *P*-values were obtained using a likelihood ratio test. Comparison between calendar periods, within each age category, was investigated using a χ^2 test.

Anti-HEV IgM prevalence and its 95% CI were estimated in those who were anti-HEV IgG–positive. This prevalence was also estimated by calendar period.

Statistical analyses were all conducted using Stata 13 (Stata Corp., College station, TX, USA). A *P*-value <0.05 was considered significant.

Results

Study population

A total of 2004 human plasma samples collected between 1996 and 2017 were considered in the analysis. Overall, the M/F ratio was 1.09 and the median age was 37 years (range: 18–91) (Table 1). Most participants (79.8%) were from Phnom Penh, the capital city of Cambodia. By calendar period, subjects of the 1996–2000 period were younger than subjects from other periods ($P < 0.001$). Furthermore, all individual in this period were from Phnom Penh, while a proportion of those from other period were not.

Anti-HEV IgG and IgM prevalences between 1996 and 2017

Between 1996 and 2017, anti-HEV IgG was detected in 824 out of 2004 samples, corresponding to an overall prevalence of 41.1% (95% CI 39.0–43.3). When analysing by calendar period of sampling, a decreasing trend of anti-HEV IgG prevalence was evidenced. This trend was more significant after gender and age standardisation (Fig. 1). The latter analysis showed that the anti-HEV IgG prevalence stood at 61.3% (95% CI 58.8–63.8) during 1996–2000 period and declined sharply to 48.8% (95% CI 48.3–49.4) by 2001–2005, then more steadily during the next periods and finally reached 32.3% (95% CI 31.9–32.7) for the 2016–2017 period.

Among the 824 plasma samples positive for anti-HEV IgG, 22 tested positive for anti-HEV IgM, yielding an overall prevalence of 2.7% (95% CI 1.6–3.8) of recent HEV infection. During the 21-year calendar period, the rate of anti-HEV IgM fluctuated, no significant difference was evidenced between calendar periods. None of these 22 subjects tested positive for both anti-HEV IgG and IgM presented detectable HEV RNA viral load.

Factor associated with the presence of anti-HEV IgG

In univariate analysis, anti-HEV IgG positivity was significantly associated with calendar period of sampling ($P < 0.001$), male gender ($P < 0.001$), age over 30 years ($P < 0.001$) and Phnom Penh residency (<0.001). In multivariate analysis, these factors remained independently associated with anti-HEV IgG positivity (Table 2).

Age-specific anti-HEV IgG prevalence by calendar period

The logistic regression analysis showed that anti-HEV IgG prevalence increased with age for all calendar periods (Table 2, $P < 0.001$). Age-specific anti-HEV IgG prevalence differed between calendar periods (Fig. 2). Overall, three patterns of prevalence were found for all age groups: (1) high prevalence in the 1996–2000 period, (2) intermediate prevalence in the calendar periods from 2001 to 2015 and (3) low prevalence in the 2016–2017 period. As compared with the 1996–2000 period, anti HEV

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Table 1. Demographic characteristic of the study population

	Total $n = 2004$	1996–2000 $n = 167$	2001–2005 $n = 632$	2006–2010 $n = 158$	2011–2015 $n = 289$	2016–2017 $n = 758$	*P* value
Gender							<0.001
Male, *n* (%)	1044 (52.1)	66 (39.5)	323 (51.1)	95 (60.1)	195 (67.5)	365 (48.2)	
Age (year)							<0.001
Median (IQR)	37 (29–49)	27 (22–36)	41 (32–50)	38 (33–44)	41 (31–56)	35 (25–51)	
Range	18–91	18–57	18–85	18–67	18–91	18–87	
Place of residence, *n* (%)							<0.001
Phnom Penh	1600 (79.8)	167 (100.0)	565 (89.4)	72 (45.6)	208 (72.0)	588 (77.6)	
Others provinces[a]	404 (20.2)	0 (0.0)	67 (10.6)	86 (54.4)	81 (28.0)	170 (22.4)	
Banteay Meanchey	7 (0.3)	–	–	–	1 (0.3)	6 (0.8)	
Battambang	19 (0.9)	–	1 (0.2)	1 (0.6)	6 (2.1)	11 (1.5)	
Kampong Cham	48 (2.4)	–	2 (0.3)	7 (4.4)	12 (4.2)	27 (3.6)	
Kampong Chnang	4 (0.2)	–	–	–	–	4 (0.5)	
Kampong Speu	16 (0.8)	–	–	–	3 (1.0)	13 (1.7)	
Kampong Thom	5 (0.2)	–	–	–	2 (0.7)	3 (0.4)	
Kampot	16 (0.8)	–	2 (0.3)	–	6 (2.1)	8 (1.1)	
Kandal	46 (2.3)	–	–	1 (0.6)	17 (5.9)	28 (3.7)	
Koh Kong	7 (0.3)	–	1 (0.2)	–	–	6 (0.8)	
Kratie	13 (0.6)	–	2 (0.3)	–	3 (1.0)	8 (1.1)	
Preah Vihear	5 (0.2)	–	–	–	2 (0.7)	3 (0.4)	
Prey Veng	21 (1.0)	–	–	–	4 (1.4)	17 (2.2)	
Pursat	6 (0.3)	–	–	–	1 (0.3)	5 (0.7)	
Ratanakiri	1 (0.05)	–	–	–	1 (0.3)	–	
Siem Reap	66 (3.3)	–	25 (4.0)	26 (16.5)	4 (1.4)	11 (1.5)	
Sihanouk Ville	90 (4.5)	–	30 (4.7)	41 (25.9)	13 (4.5)	6 (0.8)	
Svay Rieng	5 (0.2)	–	–	–	2 (0.7)	3 (0.4)	
Takeo	29 (1.4)	–	4 (0.6)	10 (6.3)	4 (1.4)	11 (1.5)	

IQR, interquartile range.
[a]other provinces include 18 out of 25 provinces of Cambodia.

IgG prevalence in the 2016–2017 period was significantly lower in age groups 18–29 years ($P < 0.001$), 30–39 years ($P < 0.001$) and 40–49 years ($P < 0.001$), but not in the group over 50 years ($P = 0.45$) (Fig. 2).

Discussion

In the current study, we analysed the temporal trends and associated risk factors of anti-HEV IgG and IgM prevalences in Cambodia during the calendar period 1996–2017. Overall, the anti-HEV IgG and IgM prevalences were 41.1 and 2.7%, respectively. We observed a significant decrease of the anti-HEV IgG prevalence over the last 21 years and a strong birth cohort effect on exposure to HEV infection in Cambodia. Indeed, risk factors independently associated with HEV infection were male gender, age above 30 years and Phnom Penh residency.

Our findings on anti-HEV IgG and IgM prevalences indicated that the Cambodian population remains exposed to HEV. This result further confirmed that HEV infection is endemic in the country. This is consistent with our previous study conducted in different populations in Cambodia, including outpatients and blood donors [10, 11]. In the present study, we report a much higher anti-HEV IgG prevalence for the period 2010–2014 than the one found in a previous investigation conducted in general population during the same time period in Siem Reap province (39.4 *vs.* 18.4%, $P < 0.0001$) [12]. This difference could be explained by the fact that Yamada *et al.* considered a younger population, which included children (median age: 29 years) [12], while only adults over 18 years were considered in our study (median age: 37 years). As previously reported in Phnom Penh and in some parts of the world such as Portugal and Germany, exposure to HEV infection in children is extremely rare [15–17]. Another study conducted more than 10 years ago in Phnom Penh reported a much lower anti-HEV IgG prevalence (5.5%). However, these data were based on an insensitive serological assay which is now obsolete [18].

Over the past two decades in Cambodia, we found that the rate of anti-HEV IgG prevalence surprisingly decreased. It is likely

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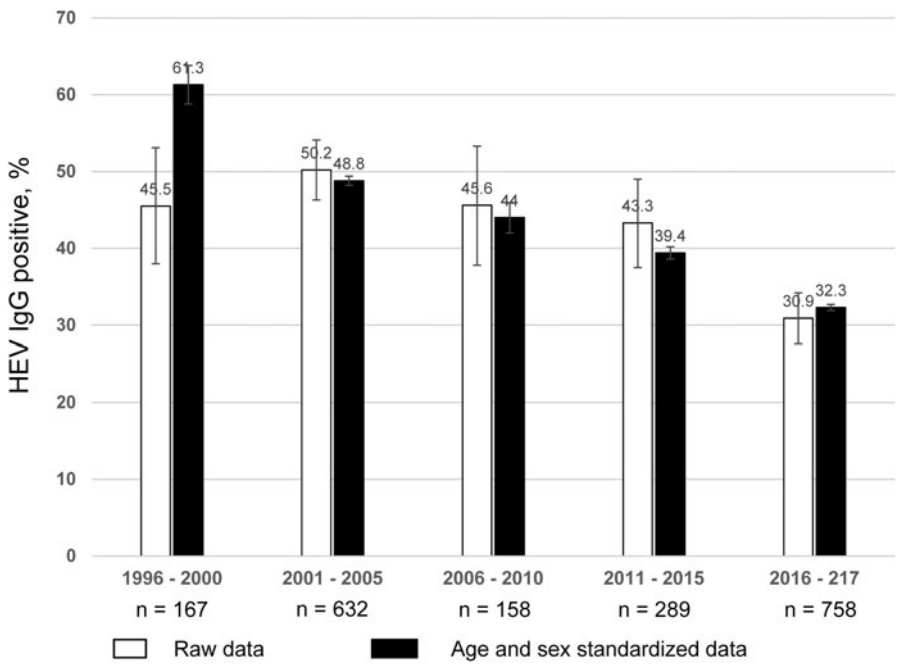

Fig. 1. Prevalence of anti-HEV IgG by calendar period among 2004 individuals collected between 1996 and 2017.

Table 2. Factors associated with anti-HEV IgG positivity

Risk factor	*N*	Subject with anti-HEV IgG *n* (%)	Crude Odds Ratio (95% CI)	*P* value	Adjusted Odds Ratio (95% CI)	*P* value[a]
Year				<0.001		<0.001
1996–2000	167	76 (45.5)	1.00 (0.64–1.54)		1.41 (0.86–2.36)	
2001–2005	632	317 (50.2)	1.20 (0.85–1.71)		0.91 (0.62–1.37)	
2006–2010	158	72 (45.6)	1		1	
2011–2015	289	125 (43.3)	0.91 (0.62–1.34)		0.59 (0.39–0.91)	
2016–2017	758	234 (30.9)	0.53 (0.38–0.76)		0.43 (0.29–0.63)	
Gender				<0.001		<0.001
Female	960	321 (33.4)	1		1	
Male	1044	503 (48.2)	1.85 (1.54–2.22)		1.90 (1.56–2.31)	
Age (year)				<0.001		<0.001
18–29	520	115 (22.1)	1		1	
30–39	573	196 (34.2)	1.83 (1.40–2.40)		1.69 (1.27–2.25)	
40–49	418	190 (45.5)	2.93 (2.21–3.89)		2.60 (1.91–3.52)	
⩾50	492	322 (65.5)	6.67 (5.05–8.81)		7.64 (5.66–10.32)	
Place of residence				<0.001		0.008
Phnom Penh	1600	692 (43.2)	1.57 (1.25–1.98)		1.46 (1.12–1.90)	
Other provinces	404	132 (32.7)	1		1	

HEV, hepatitis E virus; IgG, immunoglobulin G; CI, confidence interval.
[a]Obtained using the likelihood test.

that young generations are less exposed to HEV infection. This may be explained by improvements in sanitation conditions, food safety and access to clean water in the whole country as reported by the 2014 Cambodia demographic health survey [14]. During a similar period of time compared with our study, very few studies have monitored the temporal trends of anti-HEV IgG prevalence. In Germany, Wenzel *et al.* reported a remarkable decreased in anti-HEV prevalence from 50.7% in 1996 to 34.3% in 2011 [19]. In Denmark, Christensen *et al.* also observed decreasing trends of anti-HEV prevalence from 32.9% in 1983 to 20.6% in 2003 [20]. Nevertheless, it is difficult to compare the results of our study with those obtained from these two European countries, since the transmission mode of HEV in these two settings are different.

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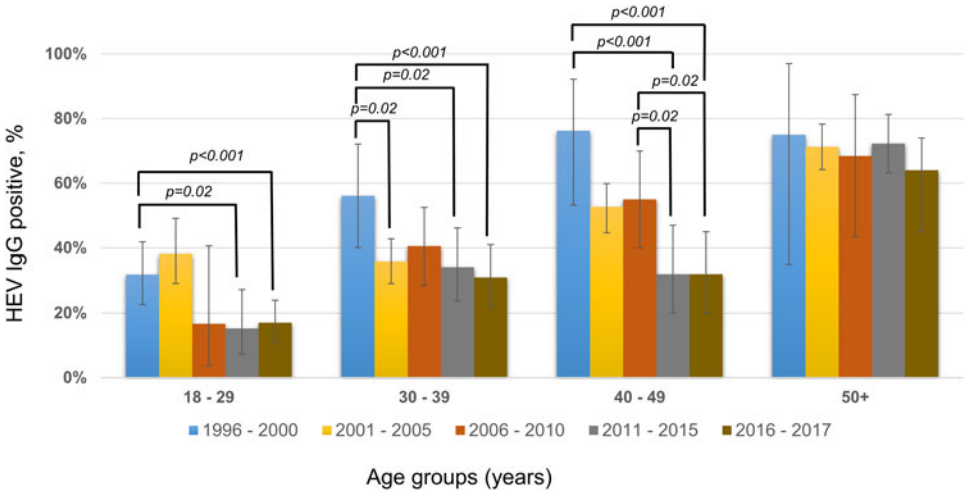

Fig. 2. Prevalence of anti-HEV IgG by age groups. Comparison between calendar periods, within each age category, was performed using a χ^2 test. Significant *P*-values ($P < 0.05$) are indicated.

The multivariate analysis demonstrated that higher risk of HEV infection was independently associated with male gender, age above 30-years and Phnom Penh residency. This could be due to the difference in lifestyles between men and women or between individuals living in the rural and urban area. In Cambodia, conservative traditions and behaviour norms for women induce inequality between women and men in terms of professional and social life [21]. Moreover, it is generally known that urban populations, in particular, men tend to be engaged in social festivities that could probably expose them to contaminated food or water more frequently than women or people living in rural area. Further investigation is needed to assess the risk of infection related to dietary behaviour and social life of the Cambodian population. When analysing by age group, for all calendar periods, anti-HEV IgG prevalence increased with age, consistent with a lifelong cumulative exposure to the virus, as described in our previous studies [10, 11] and others [20, 22, 23].

Our study has some limitations. First, the study population is not representative of the Cambodian population. However, the anti-HEV IgG prevalence could be under-estimated as the population considered in this study originated from the Institut Pasteur in Cambodia routine laboratory. This population may present a higher socio-economical condition, placing them at lower risk of HEV exposure. This limitation may prevent us from extrapolating our data of anti-HEV prevalence to the national level. The anti-HEV IgM prevalence could be overestimated in the current study since only samples positive for anti-HEV IgG were tested. Moreover, few cases of HEV recent infection with or without active viral replication may have been missed due to our strategy to assess the presence of anti-HEV IgM and HEV RNA. Another possible limitation is the heterogeneity of the study population. The specimens collected in the present study varied widely in age, gender distributions and place of residence. Finally, we were unable to retrieve and assess clinical data of participants and several risk factors already known to be associated with exposure to HEV infection such as dietary habit, occupational exposure and sexual orientation in our analysis.

In conclusion, our study shows that HEV is not an emerging pathogen, but rather seems to circulate less in Cambodia over the past two decades, in particular, in Phnom Penh. However, further investigation with more representative and homogenous population, in terms of age, gender and residency, are required.

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Conflict of interest. None.

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