Transactions of the Royal Society of Tropical Medicine and Hygiene 104 (2010) 801–805

Contents lists available at ScienceDirect

Transactions of the Royal Society of Tropical Medicine and Hygiene

journal homepage: http://www.elsevier.com/locate/trstmh

Seroepidemiology of hepatitis A and hepatitis E viruses in Aden, Yemen

Amen Ahmed Bawazir[a,b], C. Anthony Hart[c,1], Tallal A. Sallam[d], Christopher M. Parry[c], Nicholas J. Beeching[b], Luis E. Cuevas[b,*]

[a] Medical Faculty, Aden University, P.O. Box 6336, Khormaksar, Aden, Yemen
[b] Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK
[c] School of Infection and Host Defence, University of Liverpool, Liverpool L7 8XP, UK
[d] Department of Microbiology, Faculty of Medicine and Health Sciences, Sana'a University, Yemen

ARTICLE INFO

Article history:
Received 13 January 2010
Received in revised form 6 August 2010
Accepted 9 August 2010
Available online 9 September 2010

Keywords:
Viral hepatitis
Hepatitis A
Hepatitis E
Seroepidemiology
Aden
Yemen

ABSTRACT

The burden of hepatitis A (HAV) and hepatitis E (HEV) infection is unknown in Aden, Yemen. This survey describes the prevalence of antibodies against HAV and HEV among individuals attending primary health care facilities in Aden, Yemen. Five hundred and thirty eight participants, stratified by age and district population size, were enrolled and screened for anti-HAV and 356 for anti-HEV antibodies. The age-standardized seroprevalence of antibodies was 86.6% (95% CI 83.7–89.5) for anti-HAV and 10.7% (95% CI 7.5–13.9) for anti-HEV. The prevalence of anti-HAV and anti-HEV ranged from 53% and 0% in infants to 100% and 15.3% in participants >18 years old, respectively ($P < 0.001$). Viral hepatitis remains a major public health problem in Aden with trends of hyperendemicity for both infections. Priority should be given to improve water quality, sanitation coverage, and food hygiene and increase public health awareness concerning the risk of contracting infection.

1. Introduction

Despite significant achievements in recent decades to control viral hepatitis worldwide and a considerable pool of information for prevention, hepatitis A (HAV) and hepatitis E (HEV) viruses remain a significant public health problem.[1] Although these viruses share many similarities (both are ingested by mouth, spread by faecal shedding,[2] the clinical presentation cannot differentiate them and tests are mostly used in symptomatic individuals[3]) they also have considerable differences in their genomic organization and natural history. HAV is acquired early in life, while most HEV infections occur in late childhood or young adulthood.[4,5] The severity of the disease also varies and HEV infections result in high morbidity and mortality among pregnant women.[6] In addition, recent studies have suggested that HEV is zoonotic, with domestic pigs, wild boar, deer and other animals being important reservoirs,[7] which may explain some of its unique features and differences from HAV.[8]

There is no reported serologic or hybridizing cross-reactivity between HAV and HEV and serologic and nucleic acid detection tests developed for epidemiologic and diagnostic purposes have adequate sensitivity and specificity.[9]

The prevalence of anti-HAV and anti-HEV antibodies varies from 15% to 100% and 3% to 26% of adolescents, respectively,[1,10] and there is substantial evidence of a link between the reduction in the seroprevalence of HAV and HEV, mainly among children and young adults, and the improvement in living standards and sanitation.[11,12] These infections thus remain endemic in parts of the world where poor socioeconomic conditions continue to facilitate transmission,[10] while both viruses cause sporadic disease and outbreaks in industrialised countries, often linked to travel to endemic areas and contaminated food.[4,5]

* Corresponding author. Tel.: +44 151 705 7132; fax: +44 151 705 3329.
E-mail address: lcuevas@liverpool.ac.uk (L.E. Cuevas).
[1] Deceased.

0035-9203/$ – see front matter © 2010 Royal Society of Tropical Medicine and Hygiene. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.trstmh.2010.08.007

Vaccination against HAV is well established while recent trials of HEV vaccine are encouraging.[13,14]

There are no studies describing the prevalence of HAV and HEV in Aden, Yemen, even though studies in the northern governorates of the country have shown a high exposure rate to both HAV and HEV.[15,16] Historically, Aden has had a different health infrastructure to the northern governorates, with a strong emphasis in primary health care and prevention until unification with the North in 1990 and these differences may have resulted in different patterns of HAV and HEV infection. The current survey therefore describes the prevalence of HAV and HEV by age among a population attending primary health care facilities in Aden.

2. Materials and methods

This was a prospective cross-sectional survey conducted among attendants of eight polyclinics in Aden (population 590 413), Yemen. These polyclinics were selected because they were functioning on a daily basis during the time of the study. Patients attending the clinics usually reside within the same district where the polyclinic is located and attend for medical consultations and maternal and child health care. All individuals attending the clinics between April and July 2005 were eligible to participate, independent of age and gender.

A questionnaire was used to obtain information on the participants and household characteristics, medical history and known risk factors for hepatitis transmission.

The estimated sample size was calculated according to the expected prevalence of HAV and HEV in each age group to obtain a degree of precision of ± 10% according to the expected prevalence in the population. The estimated sample size for HAV required 55 children <1 year old; 55 aged 1–2 years; 58 aged 3–4 years; 96 aged 5–9 years; 90 aged 10–14 years; 92 people aged 15–44 years and 92 aged ≥ 45 years, for a total of 538 participants. Similarly, for HEV, the estimated sample size was 31 children <1 year old; 31 aged 1–2 years; 33 aged 3–4 years; 52 aged 5–9 years; 48 aged 10–14 years; 83 people aged 15–44 years and 78 aged ≥ 45 years, for a total of 356 participants. The sampling for HEV was nested within the HAV, therefore most of the 356 HEV subjects were selected from the 538 subjects studied for HAV. Therefore the enrolment of study subjects for each disease is not independent. It was, however, assumed that the two infections occurred independently, because we had no prior information to inform the potential for clustering for the two infections. In addition, samples with insufficient material for testing were replaced by the subsequent participant enrolled in the study. The number of participants enrolled ranged from 6–18 per day.

The sample size was distributed among the participating polyclinics proportional to their number of consultations by age group. Informed written consent, or oral consent for illiterate participants, was obtained from all participants, Patients were explained the purpose of the study orally and received written information to take home. Informed consent was collected following a careful explanation and providing opportunities to clarify and check the candidate's understanding. Consecutive patients were enrolled until the sample size was achieved for each age group. None of the individuals invited to participate refused and only one child was excluded due to difficulties in obtaining a suitable blood sample. The study protocol was approved by the Research Ethics Committee of the Liverpool School of Tropical Medicine, UK and the Ethical Committee in the Ministry of Public Health and Population (MOPHP) in Yemen.

Five mL of blood were obtained from all participants and serum was separated and stored at −70 °C until analysis at the Liverpool School of Tropical Medicine, UK. Samples were tested for anti- HAV and HEV antibodies using enzyme-linked immunosorbent assay (ELISA). These included ETI-AB-HAVK PLUS (N0136) Anti-HAV Enzyme Immunoassay Kits for the qualitative determination of total immunoglobulin G (IgG) antibody to human HAV (HAV-IgG) (DiaSorin S.p.A., Vercelli, Italy) and MP Diagnostic HEV ELISA kit (MP Biomedicals Asia Pacific Pte Ltd, Singapore) to detect IgG antibodies to HEV.

Data were analysed using SPSS v. 14 (SPSS Inc., Chicago, IL, USA) and included descriptive summary statistics with 95% CI for the prevalence of HAV and HEV antibodies in the population. Although sample size was estimated proportional to population size and we analyzed the data stratified by clinic, there were no differences between the clinics and it was decided to pool the data for clarity. Multivariate analysis was used to describe the association of HAV and HEV IgG antibodies and household characteristics.

3. Results

The characteristics of the 538 participants are described in Table 1. Age ranged from one month to 79 years with a mean of 18.2 years. Fifty two percent of participants were male, most were ≤18 (364, 67.7%) and 168 (31.2%) were under 5 years old. One hundred and sixteen (67%) of 174 adults did not have formal or basic education and 58 (33.4%) had secondary or higher education. Seventy two (41.4%) of 174 adults were unemployed. Participants resided with a median of 7 other household members and electricity, piped water and toilet facilities were available in 98.1%, 93.3% and 86.1% of the households respectively.

Four hundred and sixty six (86.6%, 95% CI 83.7–89.5) of 538 participants had anti-HAV IgG antibodies and 38 (10.7%, 95% CI 7.5–13.9) of 356 participants tested had anti-HEV IgG antibodies. The associations between the participants' characteristics and the presence of HAV and HEV antibodies are shown in Table 2 and Table 3, respectively. Children were less likely to have HAV and HEV IgG than adults ($p < 0.001$ and $p < 0.05$, respectively). Among children, 81% and 8% had HAV and HEV antibodies, while in adults these proportions reached 99% and 15.4%, respectively. Infants were less frequently infected with HAV than young adults (53% vs. 100%). No HEV IgG antibodies were found among infants but the levels reached 16% in adults (Figure 1).

The presence of HAV IgG antibodies was associated with age, an individual or family history of hepatitis and the availability of electricity in the household. Age

Table 1
Socio-demographic and household characteristics of the study participants

		n = 538 (%)
Age / years	mean (±S.D) [IQR]	18.2 (19.4) [3,32.3]
	Range	1 month–79 years
	children ≤18/adults/ (% children)	364/174 (67.7)
Gender	male/female (% male)	280/258 (52.0)
Education (174 adults)	basic or no formal education	116 (66.7)
	secondary and higher	58 (33.3)
Unemployed (174 adults)		72 (41.4)
Number of household members	median [IQR]	7 [5–10]
Median crowding ratio[a]	median [IQR]	3.4 [2–4]
Availability of	electricity	528 (98.1)
	piped water	502 (93.3)
	toilet	463 (86.1)

±SD = standard deviation. IQR = inter quartile range.
[a] Number of residents/number of bedrooms.

Table 2
Association of anti-hepatitis A IgG antibodies and household characteristics of the study participants

		Hepatitis A *n* = 538		Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)
		Positive (%)	Negative (%)		
Age	children <18	294 (63.1)[c]	70 (97.2)	ref.	ref.
	adults	172 (36.9)[b]	2 (2.8)	20.8 (5.1–86.1)	14.4 (3.5–59.8)
Sex	male/females (% male)	242:223 (52.0)	38:35 (52.1)	1 (0.6–1.6)	1.2 (0.7–1.9)
Education (174 adults)	basic or no formal education	115 (66.9)	2 (100)	ref.	ref.
	secondary and higher	57 (33.1)	0 (0)	undefined	undefined
Unemployed (174 adults)		66 (38.4)	2 (100)	–	–
Number of household members	Median	8	7	0.9 (0.8–1.1)	2.2 (0.9–4.8)
Median crowding ratio		3.6	3.3	0.94 (0.7–1.1)	0.8 (0.7–1)
History of hepatitis	(individual)	79 (16.9)[b]	2 (2.8)	7.3 (1.8–30.3)	6.9 (1.7–28.9)
	(family)	157 (33.7)[a]	16 (22.2)	1.8 (1.1–3.3)	1.9 (1.1–3.4)
Availability of toilet		405 (86.9)	58 (80.6)	1.7 (0.9–3.2)	1.7 (0.9–3.3)

[a] $P < 0.05$; [b] $P < 0.01$; [c] children vs. adults;.

and educational status were associated with the presence of anti-HEV IgG antibodies ($P < 0.05$). The same variables remained statistically significant after multivariate analysis.

Thirty seven (97.4%) of 38 individuals with HEV IgG antibodies had HAV IgG antibodies, while 37/314 (11.8%) participants with HAV antibodies had HEV exposure ($P = 0.1$).

4. Discussion

This is the first description of the seroepidemiological characteristics of HAV and HEV by age in Yemen. There is a significant difference in the seroprevalence between adults and children for both HAV and HEV, and differences between the two viruses. The seroprevalence of HAV antibodies in patients attending the health-centres

Table 3
Association of anti-hepatitis E IgG antibodies and household characteristics of the study participants

		Hepatitis E *n* = 356		Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)
		Positive (%)	Negative (%)		
Age	children <18	17 (44.7)	203 (63.8)	ref.	ref.
	adults	21 (55.3)[a,b]	115 (36.2)	2.1 (1.1–4.3)	2.2 (1.1–4.3)
Sex	Male: females (% male)	21:17 (55.3)	158:160 (49.7)	1.2 (0.6–2.4)	0.8 (0.4–1.6)
Education (174 adults)	basic /no formal education	11 (52.4)[b]	77 (61.6)	2.4 (1–5.8)	3.1 (1.5–6.7)
	secondary and higher	10 (47.6)	48 (38.4)	ref.	ref.
Unemployed (174 adults)		6 (28.6)	39 (31.2)	1.5 (0.5–4.1)	0.7 (0.2–1.9)
Number of household members	Median	9	8	0.9 (0.8–1.1)	1 (0.9–1.2)
Median crowding ratio		3.7	3.6	0.9 (0.8–1.1)	0.9 (0.7–1.1)
History of hepatitis	(individual)	4 (10.5)	47 (14.8)	0.9 (0.3–2.3)	1.5 (0.5–4.4)
	(family)	15 (39.5)	98 (30.8)	1.5 (0.7–2.9)	0.7 (0.3–1.4)
Availability of:	electricity	38 (100.0)	312 (98.1)	undefined	undefined
	pipe water	37 (97.4)	295 (92.8)	0.3 (0.1–2.5)	0.4 (0.1–4.9)
	toilet	33 (86.8)	271 (85.2)	3 (0.4–22.9)	1.9 (0.5–7.9)

[a] children vs. adults, [b] $P < 0.05$.

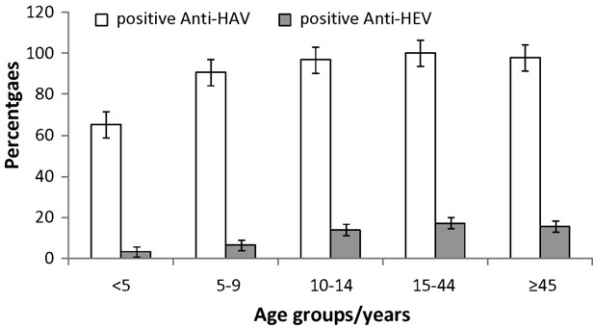

Figure 1. HAV and HEV IgG antibody prevalence by age. Bars are mean +/- SD

increased steadily from 53% among infants, 82% in pre-school children to 99% amongst adults. Although the overall prevalence was very high (86.6%), it was lower than reported by Scott et al. (99.7%) in 1988 from the northern governorates.[15] The population studied by Scott et al. was older (mean age 24.2 ±14.6 years) than the participants in this study (18.2 ± 19.4 years) and the difference could be an artefact of the population structures, use of different diagnostics and socio-demographic risk factors. The high prevalence of HAV exposure (82%) in pre-school children is similar to similar studies in developing countries such as India (86%) and South Africa (100%).[17,18] However, studies from the Middle East have reported lower prevalence rates, including Oman (53.2%),[19] Saudi Arabia (39%)[20] and Lebanon (10.5%).[12]

Most reports from the Middle East also indicate high rates of previous exposure to HAV among adults, with antibody prevalence > 95% in Egypt, Qatar, some areas of Saudi Arabia, Oman, Syria, Iran, Morocco and Algeria.[10,19,21,22] This may change with current socioeconomic development: similar rates were seen in Japan 30 years earlier (1973), while only 50% were seropositive in 2003.[11]

There were no significant differences in HAV seroprevalence by gender, education and occupational status. This might be due to improvements in the sanitary infrastructure such as piped water and sewage, which were constructed in Aden during the time of the British Colony (1839–1967), and were recently renovated and expanded. The water and sanitary coverage in the city (currently 94% and 87%, respectively) is now higher than in other cities in Yemen, e.g. water coverage is 25% and 13% in Sana'a and Taiz, respectively.[23]

There are very few reports of HEV in Yemen and one study of patients with acute hepatitis reported a positivity rate of 14%.[16] Countries are categorized into low, moderate and high HEV prevalence zones (0–2%, >2–5% and >5%, respectively)[24] and the seroprevalence rate of 10.7% obtained would place Yemen in the high seroprevalence zone, which is similar to other reports in adults in the Middle East (10–40%).[2] Population-based studies suggest that the virus is highly endemic in the region, as Egypt reported a prevalence of 30–50%, Somalia 71%, United Arab Emirates 20% and Saudi Arabia 14.8%.[24–27]

The Yemeni population has low access to potable water and sanitation with only 65% and 46% of the population having access to these services.[23] In addition, there is poor control of food hygiene, low health awareness and high illiteracy levels;[28] these factors increase the spread of HAV and HEV in the Yemeni community. Although vaccination could be desirable for the control of HAV, the WHO does not recommend the use of vaccination in populations residing in highly endemic countries, as almost all persons are infected during childhood without presenting symptoms, effectively preventing clinical hepatitis A in adolescents and adults.

The data presented however were collected in a cross sectional survey and it is not possible to account for individuals who did not survive, resulting in a selection bias towards a healthier population. A further shortcoming is the difficulty in collecting information that occurred several decades earlier, with significant recall bias of potential risk factors. In addition, although anti-HAV IgG antibodies usually persist for life, there is evidence of anti-HEV IgG antibodies becoming undetectable some years after infection or vaccination,[14] which would lead to an underestimation of the frequency of HEV exposure.

This study provides data on the prevalence of exposure to both HAV and HEV in Aden, Yemen. Both viruses are a significant public health problem, with differing rates of infection in children and adults. Exposure to HAV is common in individuals exposed to HEV, reflecting worldwide epidemiological features of these infections. Substantial changes are needed to achieve the control of HAV and HEV infection in Yemen. Priority should be given to improving water quality, sanitation coverage, food hygiene and public health awareness of the risk of contracting infection.

Authors' contributions: AAB, CAH and LEC conceived the study and developed the study protocol; CMP, TAS and NJB provided expertise on the microbiological and clinical diagnosis of viral hepatitis and data interpretation; AAB coordinated the fieldwork and enrolled all the patients in Yemen; LEC supported the implementation of the study, data analyses and report preparation; AAB and LEC prepared the manuscript with contributions from all authors. All authors contributed to, read and approved the final manuscript. LEC is guarantor of the paper.

Funding: Dr Bawazir received a scholarship from the Yemen Government to complete his PhD studies in Liverpool.

Conflicts of interest: None declared.

Ethical approval: The study protocol was approved by the Institutional Review Board of the Liverpool School of Tropical Medicine, UK and the Ethical Committee of the Ministry of Public Health and Population in Yemen.

References

1. WHO. *Hepatitis E*. Geneva: World Health Organization; 2001 WHO/CDS/CSR/EDC/2001.12.
2. Naoumov NV. Hepatitis A and E. *Medicine* 2007;**35**:35–8.
3. Bendall R, Ellis V, Ijaz S, Thurairajah P, Dalton HR. Serological response to hepatitis E virus genotype 3 infection: IgG quantitation, avidity, and IgM response. *J Med Virol* 2008;**80**:95–101.

4. Panda SK, Thakral D, Rehman S. Hepatitis E virus. *Rev Med Virol* 2007;**17**:151–80.
5. WHO. *Hepatitis A*. Geneva: World Health Organization; 2000 WHO/CDS/CSR/EDC/2000.7.
6. Chau TN, Lai ST, Tse C, Ng TK, Leung VK, Lim W, et al. Epidemiology and clinical features of sporadic hepatitis E as compared with hepatitis A. *Am J Gastroenterol* 2006;**101**:292–6.
7. Meng XJ. Hepatitis E virus: Animal reservoirs and zoonotic risk. *Vet Microbiol* 2009;**139**:253–61.
8. Tamada Y, Yano K, Yatsuhashi H, Inoue O, Mawatari F, Ishibashi H. Consumption of wild boar linked to cases of hepatitis E. *J Hepatol* 2004;**40**:869–70.
9. Myint KS, Endy TP, Gibbons RV, Laras K, Mammen Jr MP, Sedyaningsih ER, et al. Evaluation of diagnostic assays for hepatitis E virus in outbreak settings. *J Clin Microbiol* 2006;**44**:1581–3.
10. Jacobsen KH, Koopman JS. Declining hepatitis A seroprevalence: a global review and analysis. *Epidemiol Infect* 2004;**132**:1005–22.
11. Kiyohara T, Sato T, Totsuka A, Miyamura T, Ito T, Yoneyama T. Shifting seroepidemiology of hepatitis A in Japan, 1973-2003. *Microbiol Immunol* 2007;**51**:185–91.
12. Sacy RG, Haddad M, Baasiri G, Khoriati A, Gerbaka BJ, Abu-Elyazeed R. Hepatitis A in Lebanon: a changing epidemiological pattern. *Am J Trop Med Hyg* 2005;**73**:453–6.
13. Zhang J, Liu CB, Li RC, Li YM, Zheng YJ, Li YP, et al. Randomized-controlled phase II clinical trial of a bacterially expressed recombinant hepatitis E vaccine. *Vaccine* 2009;**27**: 1869–74.
14. Krawczynski K. Hepatitis E vaccine–ready for prime time? *N Engl J Med* 2007;**356**:949–51.
15. Scott DA, Burans JP, al-Ouzeib HD, Arunkumar BK, al-Fadeel M, Nigad YR, et al. A seroepidemiological survey of viral hepatitis in the Yemen Arab Republic. *Trans R Soc Trop Med Hyg* 1990;**84**:288–91.
16. Gunaid AA, Nasher TM, el-Guneid AM, Hill M, Dayton R, Pal A, et al. Acute sporadic hepatitis in the Republic of Yemen. *J Med Virol* 1997;**51**:64–6.
17. Abdool Karim SS, Coutsoudis A. Sero-epidemiology of hepatitis A in black South African children. *S Afr Med J* 1993;**83**:748–50.
18. Acharya SK, Batra Y, Bhatkal B, Ojha B, Kaur K, Hazari S, et al. Seroepidemiology of hepatitis A virus infection among school children in Delhi and north Indian patients with chronic liver disease: implications for HAV vaccination. *J Gastroenterol Hepatol* 2003;**18**:822–7.
19. MOH-SO. Viral Hepatitis A in S. Batinah. *Community Health Disease Surveil Newsl* 1999; VIII: 1–6.
20. Arif M, A-FF, Al-Frayh AR, Ramia S. Reduction in the prevalence of antibody to hepatitis A virus among young Saudi adults: implications for hepatitis A vaccine. *Saudi J Gastroenterol* 1995;**1**:93–6.
21. Tufenkeji H. Hepatitis A shifting epidemiology in the Middle East and Africa. *Vaccine* 2000;**18**(Suppl 1):S65–7.
22. Fathalla SE, Al-Jama AA, Al-Sheikh IH, Islam SI. Seroprevalence of Hepatitis a virus markers in Eastern Saudi Arabia. *Saudi Med J* 2000;**21**:945–9.
23. MOPIC. Annual Progress Report of the Poverty Reduction Strategy for 2005. Sana'a: Report of the Ministry of Planning and International Cooperation, Yemen 2006.
24. Irshad M. Hepatitis E virus: an update on its molecular, clinical and epidemiological characteristics. *Intervirology* 1999;**42**:252–62.
25. Kumar RM, Uduman S, Rana S, Kochiyil JK, Usmani A, Thomas L. Sero-prevalence and mother-to-infant transmission of hepatitis E virus among pregnant women in the United Arab Emirates. *Eur J Obst Gynecol Repro Biol* 2001;**100**:9–15.
26. Stoszek SK, Engle RE, Abdel-Hamid M, Mikhail N, Abdel-Aziz F, Medhat A, et al. Hepatitis E antibody seroconversion without disease in highly endemic rural Egyptian communities. *Trans R Soc Trop Med Hyg* 2006;**100**:89–94.
27. Abdelaal M, Zawawi TH, al Sobhi E, Jeje O, Gilpin C, Kinsara A, et al. Epidemiology of hepatitis E virus in male blood donors in Jeddah, Saudi Arabia. *Ir J Med Sci* 1998;**167**:94–6.
28. WorldBank. Yemen, Rep. Data Profile. http://devdata.worldbank.org/external/CPProfile.asp?SelectedCountry=YEM&CCODE=YEM&CNAME=Yemen%2C+Rep.&PTYPE=CP [accessed 31 July 2010].