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Seroepidemiology and risk factors of hepatitis B virus in Aden, Yemen

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Summary
Background: There is little published data concerning hepatitis B virus (HBV) infection in Aden and no data concerning risk factors for infection. This study aimed to determine the prevalence of HBV infection and risk factors for infection in Aden, Yemen.
Methods: A prospective cross sectional survey of individuals attending primary health care facilities was stratified by age and population size. Five hundred and thirty five participants were interviewed and serum was screened for the presence of Immunoglobin G HBV core antibodies (antiHBc). AntiHBc positive participants were tested for antibodies to hepatitis B surface antigen (HBsAg). A case—control analysis of risk factors for HBV was undertaken comparing risk factors between antiHBc positive cases and seronegative controls.
Results: The age-standardized seroprevalence for antiHBc was 16.2% (95% confidence interval (CI) 13.1—19.3) and for HBsAg was 1.5% (95% CI 0.5—2.5). The seroprevalence of antiHBc and HBsAg was estimated to range from 5.5% and 0% in infants to 40% and 4.6% in adults, respectively ($p < 0.001$). Age (AOR = 1.03, 95% CI = 1.01—1.05), household size (>5—9 members, AOR = 2.9, 95% CI = 1.1—7.6) and ownership of a landline telephone (AOR = 2.8, 95% CI = 1.3—5.8) were independent risk factors for HBV infection.

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Conclusions: HBV is still a public health problem in this community, with older individuals having much higher prevalence than younger generations. The results of this study would categorise Aden as a low HBV endemic zone. Perinatal transmission does not seem to be a major route of transmission.

Introduction

Hepatitis B is a serious and common infection of the liver, affecting millions of people throughout the world [1,2]. HBV infection can be complicated by chronic hepatic insufficiency, cirrhosis, hepatocellular carcinoma (HCC) and long term carriage [3]. Perinatal, child to child and parenteral transmission, transfusion of blood and its derivatives, haemodialysis, surgical interventions, dental extraction, tattooing, and wet cupping are considered as important routes of transmission in developing countries [4,5]. Additional factors include large family size, poor socioeconomic status, older age, low educational status and a history of contact with a jaundiced person [6—8]. Other risk factors such as intravenous drug use (IDU), men who have sex with men and heterosexual practices with multiple partners are more common in western countries, and infection in adolescents and young adults is the commonest mode of transmission in these settings [9,4,10].

In the Middle East region, the disease exists with different degrees of prevalence, from low to high endemicity, and prevalence varies from 2% to 20% [2,11]. Most studies conducted in Yemen to establish the prevalence of HBV (12—18.5%) have been undertaken in tertiary health care settings [11,12] and have included patients with acute or chronic hepatitis admitted to hospital [13—15] or blood donors [16]. As these populations are known to be highly selected, such studies may not reflect the real prevalence in the community and hospitalized patients often have different risk factors for infection than the general population. Unlike previous studies in Yemen, this is a community based study, which may provide more accurate information on the prevalence of HBV in the population.

A previous study in Aden among adult blood donors demonstrated a prevalence of 17.4% [16]. Two studies have also described that both vertical and horizontal modes of transmission are common in Sana'a [17,18]. Risk factors observed in these studies included blood transfusion, surgery, hospitalization, dental extraction and large family size. There have been no previous studies to describe risk factors for HBV in Aden. Therefore, this study was designed to determine the prevalence of exposure to viral hepatitis B by age among a population attending primary health care facilities in Aden City and to identify the risk factors for HBV infection in a non-hospitalized population.

Methodology

A prospective cross-sectional survey was conducted among attendants of the polyclinics in Aden City (population of 590,413), Yemen. Polyclinic users usually reside within the same district where these facilities are located and the facilities are used for medical consultation and mother and child health care. All attendants were considered eligible for recruitment, independent of gender.

A questionnaire was used to collect information on the characteristics of the participants and their households, medical history, risk factors for hepatitis transmission and general health indicators. The study participants were enrolled by age. The sample size required was estimated according to the expected prevalence of infection by age, as reported in previous surveys. The estimated sample size required was 55 participants aged <1 year; 55 aged 1—2 years; 58 aged 3—4 years; 96 aged 5—9 years; 90 aged 10—14 years; 92 aged 15—44 years and 92 participants $\geq$45 years old. These samples sizes were calculated to obtain 95% confidence intervals and a precision of $\pm$10%. The average number of participants enrolled varied from 6 to 18 per day.

A stratified design was adopted, aiming to recruit a specified number of participants in each age group in each polyclinic, proportionate to the total population served by the polyclinic. Individuals were approached when they attended the clinic for any reason in April—July 2005, and were provided with

standardized information about the study. Patients received their results according to local guidelines as soon as they became available. Written informed consent was obtained from all participants and the study protocol was approved by the Research Ethics Committee of the Liverpool School of Tropical Medicine, UK, and from the Ethical Committee of the Ministry of Public Health and Population in Yemen.

A 5 ml blood sample was obtained from 538 participants and serum was separated and stored at −70 °C until analysis. Samples were tested for anti-HBV core antibodies (antiHBc) and for hepatitis B surface antigen (HBsAg). Serum samples were tested using an ELISA for antiHBc (Bio-Rad Kit, Monalisa antiHBc PLUS, 3, Boulevard Raymond Poincare 93430 Marnes, La coquette, France) and HBsAg was detected with a Monalisa Ag HBS PLUS ELISA kit (Bio-Rad 3, Boulevard Raymond Poincare 93430 Marnes, La coquette, France). All positive samples for antiHBc were tested for HBsAg. Reactive sera for antiHBc and HBsAg were retested in duplicate. Internal positive and negative controls were included in each assay.

Data were analysed using the SPSS Version 14 for Windows. Statistical analysis included quantitative descriptive analysis and summary statistics for describing the prevalence of HBV infection (antiHBc) and carriage (HBsAg) in the studied population. Quantitative analysis of all the studied variables included 95% confidence intervals (95% CI), Chi square for trends and rates stratified by gender and other variables. For the case—control analysis, data were analysed to describe univariate associations between the characteristics of the participants and the presence of HBV. All participants with positive antiHBc were considered cases and participants without antiHBc were considered controls. Factors were investigated for participants $\leq$ or >13 years of age. Multivariate analysis was used for variables with p values <0.02 and p values <0.05 were considered statistically significant.

Results

The general characteristics of the 538 participants are described in Table 1. The age of the participants ranged from one month to 79 years with a mean of 18.2 years. Two thirds of the participants (364) were <18 years old (67.7%), 234 (43.5%) under 7 years old and 52% were male. Two thirds of the adult participants (66.6%) had no formal or basic education and 58 (33.4%) had secondary or higher education and 59% were employed. There was a median of 7 members per household and the number of residents divided by the number of bedrooms was 3.4. Electricity, piped water and toilet facilities were available in 98.1%, 93.8% and 86.7% of the households, respectively. The average time reported to reach a public clinic was a 16 min walk.

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

	$N = 538$ (%)
Age (years)	
Mean (SD) [IQR]	18.2 (19.4) [3, 32.3]
Children <18	364 (67.7)
Adults	174 (32.3)
Gender	
Male:female (% male)	280:258 (52%)
Education (adults only, $n = 174$)	
Basic/no formal education	116 (66.6)
Secondary and higher	58 (33.4)
Employment (adults only, $n = 174$)	
Employed	102 (58.6)
Unemployed	72 (41.4)
Number of household members	
Median [IQR]	7 [5—10]
Median crowding ratio[a]	
Median [IQR]	3.4 [2—4]
Availability	
Electricity	528 (98.1)
Piped water	502 (93.8)
Toilet	463 (86.7)
Average walking time to the clinic	
Mean (SD) (min)	16 (11.1)

SD, standard deviation; IQR, inter quartile range.
[a] Number of residents/number of bedrooms available per household.

Of the 538 samples tested, 87 (16%, 95% CI 13.1—19.3) were antiHBc positive and 8 (9%, 95% CI 3.1—15.3) of these were HBsAg positive. The overall carriage rate was therefore 8/538 (1.5%, 95% CI 0.7—2.8).

Individuals <18 years old had a lower frequency of antiHBc seropositivity than adults (7% vs. 35%, $p < 0.001$). Of the 62 antiHBc positive adults, 8 (12.9%) were carriers (HBsAg positive) resulting in a carriage rate of 4.6% (95% CI 2—8.9) among adults (Fig. 1).

The seroprevalence of antiHBc and HBsAg in males (18.2% and 6.8%, respectively) was similar to the seroprevalence among females (14% and 12.8%, respectively), as shown in Table 2. There was no significant difference in the prevalence of antiHBc or HBsAg by educational status and employment among adults or a history of hepatitis/jaundice in the individual or his family. A large family size though seemed to be associated with higher exposure to infection. The number of house-

Table 2 Characteristics of participants by antiHBc and HBsAg status.

	AntiHBc $N = 538$		
	Positive (%) HBsAg ($N = 87$)		Negative (%)
	Positive $N = 8$ (%)	Negative $N = 79$ (%)	$N = 451$
Age			
Children <18	0 (0)	25 (100)*	339 (62)
Adults	8 (100)	54 (87.1)	112 (38)
Male sex	3 (38)	48 (94.1)	229 (42)
Education (adults)			
Basic/no formal education	4 (50)	22 (64.7)	75 (66.9)
Employment (adults)			
Unemployed	3 (37.5)	25 (46.3)	41 (37.6)
History of hepatitis			
Individual	3 (38)	15 (19)	63 (11)
Family	1 (13)	23(29)	149 (27)
Number of household members			
1—4	0 (0)	5 (6)**	57 (10)
5—9	5 (62)	45 (57)	286 (52)
10—14	3 (38)	23 (29)	84 (15)
≥15	0 (0)	6 (8)	23 (4)
Availability			
Electricity	8 (100)	79 (100)	441 (98)
Piped water	7 (888)	77 (97)	418 (93)
Toilet	7 (88)	74 (76)	382 (85)

AntiHBc, hepatitis B virus core antibodies.
HBsAg, hepatitis B virus surface antigen.
* $p < 0.01$.
** $p < 0.05$.

hold members was greater among participants with antiHBc ($p < 0.05$) but not for those with positive HBsAg. There was no association between anti-antiHBc or HBsAg and domestic utilities.

Twenty four cases and 317 controls ≤13 years old were analysed as shown in Table 3. Among these, children with antiHBc were older than children without antiHBc (means of 6.6 and 5.4 years, respectively), but this was not significant. Household size was stratified into three categories (< 5, 5—9 and >9 members) and infection rates in subjects with an evidence of past infection increased with an increasing household size (from 8.3% to 54.2%), with an increased risk among children with >9 household members (OR = 4.7). Cases were more likely to have received blood transfusions (13% and 3%, respectively, $p = 0.05$) and to have toilet facilities (100%) than controls (82%) ($p < 0.05$). Ownership of satellites and fridges was associated with HBV infection (OR = 0.4 and 7.6, respectively). In total 63 were adult cases and 134 controls were analysed for risk factors. The mean age was higher for cases than controls (45 and 38 years, respectively) ($p < 0.01$) as shown in Table 3. Surprisingly, cases were less likely to receive blood transfusions than controls (6% and 17%, respectively) ($p < 0.05$) and were more likely to drink alcohol than controls (OR = 2.9) ($p = 0.02$). Ownership of a landline telephone was associated with HBV infection (OR = 2.8) ($p < 0.01$). Variables entered in the multivariate backward logistic regression analysis are shown in Table 3. Age increased the risk for HBV infection for each year (AOR = 1.1), household size (5—9 members, AOR = 2.9) and ownership of a land line telephone (AOR = 2.8) were associated with an increased risk of HBV infection while a his-

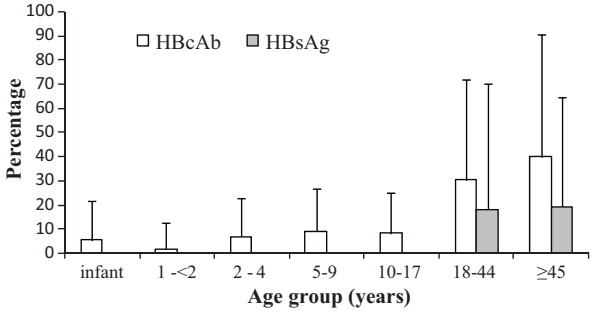

Figure 1 Seroprevalence of antiHBc and HBsAg markers by age (percentages and ±95% confidence interval).

Table 3 Univariate and multivariate regression analyses of factors associated with HBV infection.

Variable	Cases $N = 24$ (%)	Controls $N = 317$ (%)	OR	95% CI	p	AOR	95% CI	p
Age ≤ 13 years								
Mean (SD) age (years)	6.6 (4.0)	5.4 (4.1)	1.07	0.9—1.2	<0.2	1.1	1.0—1.2	<0.01
Household members								
<5	2 (8.3)	51 (16.1)	1	[a]	<0.01	1	[a]	0.05
5—9	9 (37.5)	196 (61.8)	1.2	0.3—5.6	0.8	2.9	1.1—7.6	<0.05
>9	13 (54.2)	70 (22.1)	4.7	1.1—21.9	<0.05	1.6	0.6—4.4	0.4
Blood transfusions	3 (12.5)	10 (3.2)	4.4	1.1—17.2	<0.05	0.3	0.1—0.8	<0.05
Availability of toilet	24 (100)	260 (82)	[a]	[a]	<0.05			
Ownership								
Satellite	16 (66.7)	135 (42.6)	0.4	0.2—0.9	<0.05			
Fridge	23 (95.8)	238 (75.1)	7.6	1.1—57.5	<0.05			
	Cases = 63	Controls = 134						
Age ≥ 13 years								
Mean (SD) age (years)	45.2 (13.1)	38.0 (15.5)	1.1	1.01—1.2	<0.01			
Blood transfusion	4 (6.3)	23 (17.2)	0.3	0.1—0.9	<0.05			
Drink alcohol	11 (18)	9 (6.8)	2.9	1.2—7.5	<0.05			
Ownership of landline phone	52 (82.5)	84 (63.2)	2.8	1.3—5.9	<0.01	2.8	1.3—5.8	<0.01

OR, odds ratio; AOR, adjusted odds ratio.
[a] Reference.

tory of blood transfusions (AOR = 0.3) decreased the risk.

Discussion

The overall seroprevalence of HBV infection in this study was 16% with a marked variation by age. Low rates were observed in children <18 years (7%) compared to high rates in adults (35%). Although HBV carriage had a similar distribution as HBV infection, the overall HBV carriage rate was low at 1.5%. None of the children <18 years old were carriers compared to 4.6% of the adults. This age variation reflects the cumulative exposure of adults to hepatitis. It may also reflect lack of education, inadequate blood transfusion safety measures, unsafe use of needles and syringes and lack of vaccination against HBV in previous decades. The seroprevalence found therefore is a mixture of the cohort effect of the markers of infection used, with a probably decreasing rate of infection in younger cohorts which might also be protected by the vaccine. To our knowledge there is no previous study of the seroprevalence of HBV among children in Aden to enable a valid comparison with the current situation. Most studies conducted in Yemen have reported high seroprevalence of HBV in adults in tertiary health care settings in Sana'a and have included hospitalised patients with acute or chronic hepatitis [13—15] or blood donors [16]. There have been few community surveys and only on a small scale [16,19].

The results would place Aden in the low seroprevalence zone rather than in a high endemic zone, as suggested by previous studies in the Middle East [11,12]. In addition, the testing strategy to test antiHBc first, and then positive antiHBc samples being further tested for HBsAg may have resulted in an under-representation of carriers who had lost the antiHBcs in this population. For example, the 17.4% prevalence reported by Al-Nassiri and Raja'a [14] was based on antiHBc+/HBsAg cases. If those who were HBsAg positive were also included, the seroprevalence would be 24.1% as the HBsAg+ rate was 6.7%. Further representative studies in other areas of the country including all age groups, therefore could be conducted to confirm a low HBV endemicity in Yemen. Age is an important confounder in the distribution of HBV infection in this community. Previous studies conducted among hospital employees [20] and blood donors [21] in Aden reported high rates of infection among adults and resembled the findings of this study in adults. The low seroprevalence among children that increases in early adolescence supports the hypothesis that HBV infection is more likely to be acquired in early adulthood rather than by vertical transmission. It should be noted however that recent studies in pregnant women in the perinatal period attending primary health care facilities in Aden City [22], showed a carriage rate of 2.8% [18].

Similar rates were reported from Sana'a, where 4% of healthy mothers were identified to be carriers. Vertical transmission therefore may not be a rare event.

Therefore the lower prevalence of HBV found in this study compared to previous studies is most likely due to the inclusion of children, as previous studies were in adults. Adults in this study had a sero-prevalence rate of 40% and a carriage rate of 4.6%, which are very comparable to previous studies. In addition, this study demonstrates that HBV infection in Aden is mainly acquired later in life rather than through perinatal transmission.

The provision of the HBV vaccine within the EPI since 2000 seems to have had a positive impact in the prevention of HBV infection among children. The low frequency of infection among children may also reflect a reduction in post-transfusion-related infections through the use of safer blood supplies and more widely practiced infection control procedures among medical personnel in recent years.

Four factors were significantly associated with HBV infection. These were age, family size, use of blood transfusions or blood products and having a landline telephone. Even after stratification into age groups $\leq$ and >13 years, age was still positively associated with HBV infection in adults. This suggests that new infections can occur in adults. Some studies have shown that HBV infection is more likely to occur within large families, suggesting that person to person transmission can occur in these conditions through common utensils shared by the household members [6,23]. In Yemen however, Scott and Al-Nassiri found no association between HBV infection and household size in Sana'a. This difference could be due to variation in the study design and population settings [14,17]. The reason for association of intra-familial transmission with family size could have been complemented by data on viral activity through testing HBsAg positive subjects for HBeAg alone or, preferably, along with qualitative and/or quantitative measurements of HBV-DNA. Unfortunately it was not possible to obtain these data and this should be considered a study limitation. This could provide clues on the apparently efficient intra-familial transmission.

Surprisingly, a history of receiving blood transfusions had an inverse association with HBV infection in adults. This finding is inconsistent with previous studies from Sana'a [14,17,18]. However, a previous study on HBV markers among blood donors identified a lower rate of carriers in Aden than in Sana'a and may explain these paradoxical findings [16]. Blood services in Yemen rely mainly on volunteer blood donation, usually by friends or relatives and sometimes through donation campaigns. The transmission of HBV via blood transfusion in Yemen has become less frequent than in the past due to the implementation of control measures in blood transfusion facilities [24]. The association between ownership of a landline telephone and HBV infection is difficult to explain. This is a surrogate marker of wealth in this setting, suggesting that cases were economically better off than controls. However, it is possible that the more wealthy individuals had more access to health care and thereby had a higher exposure to unsafe injections.

There are limitations in the data presented here. As the surveys were cross sectional, it was not possible to account for infected individuals who did not survive, whether neonates, babies, children or adults, resulting in a selection bias toward a healthier population. A further shortcoming is the difficulty in collecting information that had potentially occurred several decades earlier and recall bias is likely to play a significant role. Finally, it is difficult to collect information concerning highly sensitive cultural issues (e.g. information on multiple sexual partners, the use of recreational drugs and alcohol) in this environment.

The findings from this study therefore highlight that HBV is still an important public health problem in this community, although the trend of infection seems to have improved with the reduction in blood transmitted infections and increase in immunisation in the last decades. None of the children <18 years had evidence of HBV carriage suggesting that perinatal transmission is not a major mode of transmission for HBV in this sitting. The overall seroprevalence of HBV would define Aden as a low endemic zone. Further studies in other areas of the country with inclusion of all age groups, could provide further evidence on HBV endemicity in Yemen. Although factors such as multiple sexual partners, intravenous drug use and contact with sex workers are not frequently reported in Yemen, qualitative studies would be needed to obtain more reliable information on these factors.

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

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical approval

The study protocol was approved by the Research Ethics Committee of the Liverpool School of Tropical Medicine, UK, and from the Ethical Committee of the Ministry of Public Health and Population in Yemen.

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