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EPIDEMIOLOGY OF HEPATITIS B IN THE GEZIRA REGION OF SUDAN

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# EPIDEMIOLOGY OF HEPATITIS B IN THE GEZIRA REGION OF SUDAN

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Abstract. To determine the prevalence of and risk factors for hepatitis B infection in rural Sudan, 2 villages in the Gezira were surveyed. There were 851 subjects (age 1-89 years; mean age 24.6 years) of equal sex distribution, 408 from Khalawaat and 443 from Saleim. HBsAg was found in 18.7%, and seropositivity for any hepatitis marker (HBsAg, anti-HBs, or anti-HBc) was found in 63.9%. The prevalence of HBsAg was highest in subjects <5 years of age (32.3%). Seropositivity for any hepatitis marker increased from 48.4% in subjects <5 years to 88.5% in persons  $\geq$  50 years of age. HBeAg was present in 70% of HBsAg-positive women of childbearing age. Residence in Khalawaat and parenteral therapy for malaria were found to be independent risk factors for HBsAg-positivity. Age, residence in Khalawaat, crowding, and having had a tattoo were predictive of seropositivity for any hepatitis marker. The reason for increased markers of hepatitis B in Khalawaat compared to Saleim was not apparent.

Of adults in Africa, >50% may have serologic evidence of past hepatitis B infection.<sup>1,2</sup> The predominant modes of transmission of hepatitis B in Africa are not known. Infection occurs at an early age, but maternal-infant transmission does not appear to be as important as in Southeast Asia.<sup>3-5</sup>

The prevalence of hepatitis B infection in Sudan is also high. Hepatitis B antigenemia is found in 19% of some groups. Again, the major modes of transmission are not known.<sup>6–8</sup> The present study determined the prevalence and risk factors of hepatitis B infection in 2 villages in the Gezira.

#### MATERIALS AND METHODS

#### Patient population

Study subjects were from the villages Khalawaat and Saleim, which are approximately 60 km apart. The Gezira region is between the White and Blue Nile south of Khartoum in the northern, arid, and predominantly Moslem region of Sudan. It is the principle agricultural area of this country. An extensive system of man-made canals provides irrigation for farming. A network of unpaved roads connects villages which are engaged mainly in agricultural activities.

These 2 villages were selected because their

differing sizes and health systems offered contrasts which could help determine modes of hepatitis B transmission. Khalawaat has approximately 1,000 inhabitants. A 1 room "dressing station" staffed by a nurse with no technical training is the only source of local health care. The station contains few drugs and no diagnostic equipment.

Saleim has approximately 3,000 inhabitants. Health care is provided in a dispensary staffed by a technician with 2 years of training in the diagnosis and treatment of common infections. A microscope is present for the identification of malaria and stool parasites. Antimalarial and antischistosomal drugs are available but in short supply.

Living conditions in the 2 villages are similar. Houses are constructed of mud brick, and animals are kept within family compounds adjacent to dwellings Electricity is supplied intermittently to a few buildings by gasoline generators.

Water is provided in both villages by wells. Some residents obtain water directly from the wells manually; others have access to water piped to various areas from a central elevated holding tank. Outdoor dry pits are used for sewage disposal in nearly all homes.

The populations are stable, although adult men will often have worked for brief periods in other areas of the Sudan. It is unlikely that any study subjects ever visited the other village.

# Statistics

Field survey

Each village was surveyed on 3 consecutive days during December 1986. The project was explained to the village elder, and his consent was obtained. All villagers appearing at the study site were evaluated after individual informed consent was obtained. It was not possible to evaluate subjects grouped by household. Subjects were included on the basis of time of appearance. Selection bias cannot be discounted.

Three Sudanese physicians initially interviewed participants and completed a questionnaire. Basic demographic data, including age, sex, occupation, home water source, family size, and the number of rooms in the home, were elicited. A crowding index was calculated for each person in the study by dividing the number of household members by the number of rooms in the home. Study subjects were asked about potential risk factors for hepatitis B transmission, including blood transfusions, hospitalizations, childbirth, dental work, tattoos, and the number and types of treatment for schistosomiasis and malaria. A history of jaundice or contact with jaundiced household members and friends was also noted. Subjects were examined only for jaundice.

Seven ml or less of blood was drawn from each subject. Samples were kept cool with icepacks until centrifuged. Serum samples were then frozen at  $-20^{\circ}$ C. Each subject was provided with containers for stool and urine samples. All subjects found positive for schistosomiasis were treated with praziquantel.

#### Laboratory

Sera were tested for HBsAg, anti-HBs, anti-HBc. anti-delta (anti-HD), and e antigen (HBeAg) using enzyme-immunoassay test kits (Abbott Laboratories, North Chicago, IL). Samples were first tested for HBsAg. If positive for HBsAg, samples were further tested for anti-HD and HBeAg. If negative for HBsAg, samples were tested for anti-HBs and subsequently for anti-HBc if negative for anti-HBs.

Stools were examined for Schistosoma mansoni eggs using a modified Kato method.<sup>9</sup> Urine was examined for S. haematobium.<sup>10</sup> Univariate comparisons were performed using the chi-square test with Yates' correction for proportions and the Student's *t*-test for continuous variables. To identify independent associations, stepwise (forward and backward) unconditional multiple logistic regression analysis was performed using the True Epistat software package (Epistat Services, Richardson, TX). For data analysis of hepatitis markers, subjects were categorized as antigen-positive (subjects positive for HBsAg, seropositive (subjects positive for any of 3 markers: HBsAg, anti-HBs, or anti-HBc), or seronegative (subjects negative for all hepatitis markers). Mean values were reported as  $\pm 1$  SD. Significance was designated at P < 0.05.

#### RESULTS

### Patient population

There were 851 subjects. The mean age was 24.6  $\pm$  17 years. Fifty-one percent were male (mean age 25.3  $\pm$  18.7 years) and 49% were female (mean age 23.8  $\pm$  15.1 years). Approximately 50% of the inhabitants of Khalawaat (408 individuals, age 1-89 years, mean age 23.4  $\pm$  17.1 years) were sampled. Approximately 15% of the inhabitants of Saleim (443 individuals, age 1-81 years, mean age 25.7  $\pm$  16.9 years) were sampled. The male-to-female ratios were similar.

The socioeconomic standards of the 2 communities were comparable. Subjects lived in mudbrick houses with an average of 7.8 people in 2.8 rooms in Khalawaat (crowding index  $3.2 \pm 1.7$ ) and 7.9 people in 3 rooms in Saleim (crowding index  $3.0 \pm 1.7$ ). Goats, chickens, and donkeys were kept by a similar percentage of families from each village.

Subjects were generally healthy and none were jaundiced. There was no evidence of protein-calorie malnutrition.

#### Prevalence of hepatitis markers

Hepatitis B surface antigen was found in 159 subjects (18.7%). Additionally, anti-HBs was found in 247 and anti-HBc in 138. When all markers were considered, 544 (63.9%) subjects were seropositive. In Khalawaat, 22.3% were HBsAg positive and in Saleim, 15.3% were pos-

	TA	BLE I		
Hepatitis markers	in	Khalawaat	and	Saleim

Percent	positive
Khalawaat	Saleim
22.3 (91/408)	15.3 (68/443)
34.4 (109/317)	36.8 (138/375)
34.1 (71/208)	28.3 (67/237)
66 4 (271/408)	61 6 (273/443)
26.3 (15/57)	29.3 (17/58)
58.7 (37/63)	74.4 (32/43)
	Percent Khalawaat 22.3 (91/408) 34.4 (109/317) 34.1 (71/208) 66.4 (271/408) 26.3 (15/57) 58.7 (37/63)

itive (P = 0.012). Seropositivity for any hepatitis marker was found in 66.4% of subjects living in Khalawaat and 61.6% in Saleim (P = 0.17; Table 1).

The prevalence of HBsAg-positivity was highest (32.3%) in subjects <5 years, but then remained fairly constant as age increased. In contrast, seropositivity for any hepatitis marker was related to advancing age, increasing from 48.4% in subjects <5 years to 88.5% in those  $\geq 50$  years (Fig. 1). The most marked increase in seropositivity was observed in study subjects 20-29 years old. No children <1 year were tested.

Age-specific analysis of hepatitis B markers demonstrated a higher HBsAg and seropositivity rate in Khalawaat than in Saleim for most age groups (Table 2). A higher percentage of men than women were positive for HBsAg (19.4% vs. 17.9%) and a higher percentage of men were seropositive for any hepatitis marker (66.0% vs. 61.7%). However, these differences were not statistically significant.

Age-specific analysis demonstrated similar rates of infection for both men and women. There was no association in either village between the frequency of hepatitis markers and a person's primary occupation or home water source.

Of 115 HBsAg-positive samples tested for delta antibody, 27.8% were positive. No significant difference in prevalence of anti-HD between the 2 villages was observed (Table 1). The mean age of anti-HD positive and negative subjects was similar (25.5 vs. 24.1 years).

In 106 HBsAg-positive sera, HBeAg-positivity was comparable in Saleim (74.4%, 32/43) and Khalawaat (58.7%, 37/63; P = 0.15). In women 16-45 years old who were tested, the percentage positive for HBeAg was 76.5% (13/17) in Saleim and 61.5% (8/13) in Khalawaat (P = 0.63).

# Schistosomiasis infection

Of 822 stool samples and 794 urine samples. 380 (46.2%) were positive for S. mansoni and 12 (1.5%) for S. haematobium. The mean egg count for S. mansoni infected persons was 615  $\pm$  1.041 eggs/gram, and S. haematobium subjects averaged 21  $\pm$  23 eggs/10 ml urine.



FIGURE 1. Comparison of the age-specific prevalence rates of HBsAg (lower 2 curves) and seropositivity (upper 2 curves) between females (-----) and males (-----).

	Percent HB	sAg positive	Percent se	ropositive
Age (years)	Khalawaat	Saleim	Khalawaat	Saleim
1-4	41.2 (7/17)	21.4 (3/14)	64.7 (11/17)	28.6 (4/14)
5-9	24.6 (15/61)	13.3 (6/45)	47.5 (29/61)	42.2 (19/45)
10-14	21.3 (20/94)	11.5 (7/61)	55.3 (52/94)	44.3 (27/61)
15-19	21.7 (10/46)	13.8 (11/80)	56.5 (26/46)	45.0 (36/80)
20-29	21.1 (15/71)	20.8 (20/96)	74.6 (53/71)	63.5 (61/96)
30-39	16.7 (7/42)	15.4 (8/52)	83.3 (35/42)	73.1 (38/52)
40-49	18.5 (5/27)	19.5 (8/41)	88.9 (24/27)	90.2 (37/41)
≥ 50	24.0 (12/50)	9.3 (5/54)	82.0 (41/50)	94.4 (51/54)
Totals	22.3 (91/408)	15.4 (68/443)	66.4 (271/408)	61.6 (273/443)

 TABLE 2

 Comparison of age-specific prevalence of HBsAg and seropositivity for HBsAg, anti-HBs, or anti-HBc in Khalawaat and Saleim

Twenty-eight percent of the subjects from Khalawaat and 72.2% from Saleim had been treated for schistosomiasis, and 86% from Khalawaat and 79.5% from Saleim had been treated for malaria. Treatment using parenteral medications was very common. Injections had been received by 14.8% of the subjects for schistosomiasis infection and by 69.5% for the treatment of malaria. Only 8 (2.5%) individuals  $\leq$  15 years had received parenteral antischistosomal therapy. There was no difference between the 2 villages in the number of injections received by study subjects.

## Risk factors

Univariate analysis suggested an association between the presence of HBsAg and 2 risk factors: residence in Khalawaat and infection with S. mansoni. When seropositivity for all hepatitis markers was analyzed, associations were noted for crowding, hospitalization, dental treatment, tattoos, jaundice, infection with S. mansoni, and parenteral therapy (Table 3).

Because the prevalence of hepatitis markers and the occurrence of many potential risk factors of infection were associated with age, multiple stepwise logistic regression analysis was used to identify independent associations. Residence in Khalawaat and parenteral therapy for malaria were found to be significantly associated with HBsAg-positivity. Age, residence in Khalawaat, crowding, and a tattoo were found to be independently predictive of seropositivity for any hepatitis marker (Table 4).

There was no association between positivity for delta antibody and any potential risk factors. Delta-positive study subjects did not report a history of clinical jaundice more frequently than others.

#### DISCUSSION

The high prevalence of HBsAg in this study is comparable to that of the most highly endemic areas of hepatitis B infection in Southeast Asia, Ethiopia, and Somalia.<sup>1,11-13</sup> Half of this study population had been infected by 4 years of age, and most of the population had markers of hepatitis B infection by the age of 40.

Most of the subjects positive for HBsAg can be assumed to be chronic antigen carriers since none were acutely ill or jaundiced, and HBsAg detected in cross-sectional surveys generally indicates chronic infection.<sup>16-17</sup> The risk of acquiring chronic hepatitis B appeared to be greatest in the young. A similar pattern of early childhood infection is found in Southeast Asia, where a large percentage of chronic carriers results from perinatal transmission.18 Perinatal transmission could also account for substantial infection and resulting chronic antigenemia in this Sudanese population. The high proportion of women of child-bearing age who were e antigen positive lends support to this prospect.<sup>19</sup> Alternatively, early horizontal transmission within families could account for this pattern of infection as in other parts of Africa.3.5

A high prevalence (28%) of delta infection among HBsAg-positive study subjects was found. Delta infection has been found to be a cause of acute hepatitis in nearby Khartoum and other adjacent countries.<sup>8,20</sup> Association between a history of jaundice and delta infection was not detected. The reason is not apparent from the data.

		Perce	ent HBsAg positive		1	Percent seropositive	
Vanable	Variable pr	esent	Variable absent	P value	Variable present	Variable absent	P value
Khalawaat resident	22.3 (91)	(408)	15.3 (68/443)	0.012	66.4 (271/408)	61.6 (273/443)	0.166
Male sex	19.4 (85/	(438)	17.9 (74/413)	0.639	66.0 (289/438)	61.7 (255/413)	0.224
Crowding index*	19.6 (82)	(418)	17.8 (77/432)	0.56	67.5 (282/418)	60.7 (262/432)	0.046
History of:					-		
Hospitalization	19.7 (55/	(279)	18.2 (104/572)	0.657	70.6 (197/279)	60.7 (347/572)	0.006
Dental treatment	19.5 (33)	(169)	18.5 (126/682)	0.839	76.3 (129/169)	60.9 (415/682)	< 0.001
Transfusion	16.2 (6/	(37)	18.8 (153/814)	0.859	70.3 (26/37)	63.6 (518/814)	0.518
Tattoo	22.3 (40)	(179)	17.7 (119/672)	0.191	78.2 (140/179)	60.1 (404/672)	<0.001
Jaundice	18.7 (34)	(182)	18.6 (124/668)	1.000	70.3 (128/182)	62.1 (415/668)	0.051
Contact with jaundiced person	20.2 (107)	(183)	16.3 (52/319)	0.193	64.8 (344/531)	62.7 (200/319)	0.589
Infection with:							
S. mansoni	22.4 (85/	(380)	16.5 (73/442)	0.042	67.4 (256/380)	60.6 (268/442)	0.054
S. haematobium	16.7 (2)	(12)	19.7 (154/782)	1.000	83.3 (10/12)	63.8 (499/782)	0.273
Parenteral therapy for malaria	17.3 (102/	(165)	21.9 (57/260)	0.13	66.8 (395/591)	57.3 (149/260)	0.01
for Schistosomiasis	20.6 (26/	/126)	18.3 (133/725)	0.628	79.4 (100/126)	61.2 (444/725)	<0.001
* Considered present if a median crowding in	idex of 2.75.						

Univariate analysis of HBsAg and seropositivity for any hepatitis marker in all study subjects

TABLE 3

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#### TABLE 4

Significant independent predictors of HBsAg and seropositivity by stepwise multivariate logistic regress on analysis

Variable	B-coefficient	P value	Odds ratio	95% confidence interva
Antigen positivity (HBsAg)				
Residence in Khalawaat	0.49	0.009	1.64	1.15 to 2.33
Injections for malaria*	0.03	0.022	1.03	1.00 to 1.06
Seropositivity				
Age (per year)	0.05	< 0.001	1.05	1.04 to 1.07
Residence in Khalawaat	0.33	0.032	1.39	1.03 to 1.88
Crowding (2 groups)†	0.40	0.007	1.49	1.10 to 2.02
Tattoo (2 groups)	0.46	0.028	1.58	1.04 to 2.39

Odds ratio based on each additional injection for malaria.
† Continuous variable "crowding" divided at the median.

For many subjects, anti-HBc was the only indication of prior infection. The reason for the variance between anti-HBs and anti-HBc positivity was not apparent. Finding anti-HBc as a single marker was not related to the age or sex of study subjects. Other studies have found a large percentage of individuals positive for anti-HBc but negative for anti-HBs.<sup>2, 21, 22</sup>

Several risk factors for hepatitis B transmission, including medical injections, tattooing, and crowded living conditions, were found to be independently associated with hepatitis markers by multivariate analysis. These risk factors have been previously identified as potentially significant in developing countries.<sup>23, 24</sup> The possible spread of hepatitis B by medical injections has been noted before.<sup>4</sup> The relationship between the crowding index and seropositivity could indicate increased hepatitis B transmission due to crowding, or it could indicate other risk factors associated with a lower standard of living.<sup>25</sup>

The reason for the increased risk of hepatitis B infection in Khalawaat could not be determined. No specific difference in lifestyle, standard of living, or health care was found.

Schistosomiasis infection was not found to be a risk factor for hepatitis B. Similar results have been seen in Egypt.<sup>26</sup>

A very high level of hepatitis B infection was found in these 2 villages. Although the exact mechanisms of transmission could not be determined, the initially high level of infection in the youngest study subjects indicates that significant transmission occurs early in life, followed by a steady continuation of exposure in adults of both sexes.

Informed consent was obtained from all study subjects or their parents. Guidelines of the NAMRU-3 Committee for the Protection of Human Subjects were followed.

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