

## Different seroprevalences of antibodies against *Neisseria meningitidis* serogroup A and *Haemophilus influenzae* type b in Sudanese and Swedish children

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### SUMMARY

Sampling of sera from 202 Sudanese and 124 Swedish children 1–14 years of age was conducted at the end of the 1980s presenting an opportunity to compare the seroprevalence of anti-*Neisseria meningitidis* (MC) serogroup A antibodies in an area immediately before outbreak of an epidemic (Sudan 1988) with a low endemic area (Sweden). An ELISA antibody assay was developed for detection of antibodies against capsular polysaccharide of MC serogroup A and *Haemophilus influenzae* type b (Hib). Serum antibody against MC serogroup A was found significantly more frequently in Sudanese than in Swedish children. This indicates that factors other than herd immunity, as measured by serum antibodies against MC serogroup A polysaccharide, are important for avoidance of an MC serogroup A epidemic. The seroprevalence of Hib antibodies was, in contrast, significantly higher in Swedish than in Sudanese children, especially for 5–9-year-old children. A possible explanation may be the different systems of day-care of children in the two countries.

### INTRODUCTION

Acute bacterial meningitis (ABM) continues to be a worldwide health problem with severe morbidity, significant mortality and a high incidence of residual sequelae in survivors [1–4]. The problem is greater in many developing countries with peak incidences of several hundred per 100 000 person years [5, 6] compared with many developed countries with peaks up to some tens per 100 000 person years [2, 7, 8]. The toll of disease is highest among children. The distribution of the three most important causative organisms for ABM, *Haemophilus influenzae* type b (Hib), *Neisseria meningitidis* (MC) and *Streptococcus pneumoniae* (PNC), varies in different populations. However, in both Sudan and Sweden Hib accounts for more than half of the cases of ABM among children in the non-epidemic situation [9, 10] with a documented increased incidence in Sweden since 1970 [9]. Reliable data concerning the incidence of Hib meningitis in Sudan are lacking. The high

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proportion of Hib in both countries is followed by MC which accounted for 27% of the cases in Sweden [9] and 18% in Sudan [10]. In Sweden PNC caused less than 10% of ABM among children but was higher, 16%, in Sudan [9, 10].

Epidemics of MC have been reported from all continents during the 1970s [11]. Among the MC, serogroup A strains are the leading cause of epidemics [12] which have been most extensive in the 'meningitis belt' of Africa including Sudan [12-14]. However, Sweden has not experienced an epidemic since the 1940s and the mean annual incidence 1956-80 was 5.4 per 100000 children below 5 years of age [9] and during the 1980s it was 4.4 per 100000 [15]. During the 1980s few MC serogroup A strains have been isolated in Sweden [15] comprising 0-5% of invasive MC strains each year.

Just before the 1988 epidemic in Sudan, caused by *N. meningitidis* serogroup A, ET 5, clone III-1, sulphonamide-resistant [14], sampling of sera from Sudanese children was conducted. Sampling of sera from Swedish children was performed during 1989-90. This gave the opportunity to compare the seroepidemiology of anti-MC serogroup A antibodies in an area immediately before the outbreak of an epidemic (Sudan) with a low endemic area (Sweden) in which the same MC serogroup A, ET 5, clone III-1, sulphonamide-resistant strain had been isolated in 1987 without causing an epidemic [14].

Epidemics caused by Hib have not been reported, but invasive Hib infections are endemic throughout the world [16-19]. In children younger than 5 years the incidence of invasive Hib disease has been reported to vary between 40 and 130 per 100000 person years [16-19]. Both socioeconomic and genetic factors have been suggested as reasons for this variation [18, 20]. Available reports and experiences of Hib meningitis indicate no major difference concerning the impact of the disease between Sudanese and Swedish children. Only scattered vaccinations have been conducted in the two countries. Great differences in habits and socioeconomic levels can however be expected to influence the epidemiological situation of Hib.

Our hypothesis was that herd immunity, as measured by anti-MC serogroup A antibodies, was lower in the Sudanese population just before an epidemic than in the population in which the incidence of meningococcal disease did not increase. In addition the aim with the present study was to elucidate how the response to Hib developed with age in the two unvaccinated populations.

## MATERIAL AND METHODS

### *Serum samples from Sudanese children*

A total of 202 Sudanese children 1-14 years of age was recruited for the study. Blood samples were taken from 122 infants and young children (1-4 years) who were admitted to two hospitals in Greater Khartoum for elective minor surgery. None of them showed any sign of infection at the time of sampling. For the age group 5-14 years, blood samples were obtained from different primary, intermediate and secondary schools in Khartoum. Vaccinations with MC A+C vaccine (different manufacturers) had been conducted annually 1984-7. The state of immunization was ascertained for each individual. No vaccination for Hib disease had been performed in the children studied.

Table 1. Number of non-vaccinated children with serum anti *MC* serogroup A and *Hib* antibodies of immunoglobulin classes G, A and/or M

Number of positive Ig classes per child	<i>N. meningitidis</i>				<i>H. influenzae</i>			
	Sudan		Sweden		Sudan		Sweden	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
0	26	15	33	26	42	25	36	29
1	64	38	42	34	59	35	19	15
2	40	24	33	26	33	20	33	27
3	38	23	16	13	34	20	36	29
Total	168		124		168		124	

About 10 ml of venous blood was drawn, after obtaining informed consent, and separated within 3–4 h. The collected sera were stored in aliquots of 1 ml at  $-20^{\circ}\text{C}$  and transported to Sweden on dry ice where they were stored at  $-70^{\circ}\text{C}$  till the time of assay.

#### *Serum samples from Swedish children*

Serum samples from 124 children 1–14 years of age were collected in 1989–90 at the Department of Pediatric Anaesthesia, St Görans Hospital, Stockholm, Sweden as described previously [21]. From each child 2–3 ml blood was drawn, the serum collected and stored in aliquots of 1 ml at  $-70^{\circ}\text{C}$  before use. No Swedish child had been vaccinated with meningococcal or Hib vaccine.

#### *Antibody assays*

Anti-*N. meningitidis* serogroup A and anti-*H. influenzae* type b polysaccharide antibodies of IgG, IgA and IgM were assayed with an ELISA-method as follows.

Microtitre plates (Nunc Maxisorp, Nunc Inter Med, Denmark) were precoated with 100  $\mu\text{l}$  poly-L-lysine per well (1.25 mg/l PBS, Sigma Chemical Co., St Louis, USA) for the IgG and IgA assays. For the IgM assay no precoating was performed. The precoated plates were incubated for 5 h at room temperature and washed with PBS. Meningococcal serogroup A polysaccharide (Institut Mérieux, Lyon, France) or *H. influenzae* type b polysaccharide (Connaught, Canada) was coated (100  $\mu\text{l}$ /well of 2.5 mg polysaccharide/l carbonate buffer pH 9.6) to half of the wells in a plate (the other half had only carbonate buffer) overnight at  $4^{\circ}\text{C}$ . Thereafter the plates were washed with PBS + 0.05% Tween 20 and post-coated with 10% fetal calf serum (FCS, Flow Laboratories, Irvine, Scotland) in PBS for 60 min at  $37^{\circ}\text{C}$ . After washing, 100  $\mu\text{l}$  of serum diluted 1/200 in 10% FCS/PBS was added and incubated for 60 min at  $37^{\circ}\text{C}$ , washed (PBS-Tween) and 100  $\mu\text{l}$  conjugate (goat anti-human IgG, A and M respectively conjugated to ALP, Sigma) diluted 1/1000 in 10% FCS/PBS added and incubated for 60 min at  $37^{\circ}\text{C}$ . After washing 100  $\mu\text{l}$  of substrate (paranitrophenylphosphate, Sigma in diethanolamin, 1 g/l) was added and incubated for 60 min at room temperature after which the absorbance was read at 405 nm. All samples were run in duplicate, with and without antigen-coated wells. The difference between the mean absorbance value in coated and uncoated wells was related to the absorbance value of a reference

serum included on each plate to calculate the antibody concentrations of each sample.

The reference was a 14 days post-revaccination serum (Vaccin meningococcique A+C, Institut Mérieux, and Haemophilus b conjugate vaccine, proHIBit<sup>®</sup>, Connaught Laboratories, Swiftwater, USA). The total anti-MC A polysaccharide antibody concentration was 106  $\mu\text{g/ml}$  (IgG 476 U/ml, IgA 913 U/ml and IgM 39 U/ml when referred to a WHO reference serum with 100 U/ml by Helena Käyhty, Helsinki, Finland). The total anti-Hib polysaccharide antibody concentration was 81  $\mu\text{g/ml}$  of which IgG was 61  $\mu\text{g/ml}$  and IgA 16  $\mu\text{g/ml}$ . For anti-Hib polysaccharide IgM reference a high-titre clinical serum was used which unfortunately was spent before quantification of the antibodies.

#### *Statistical methods*

Differences in frequency distributions were tested with the chi-square test of homogeneity. The antibody levels were analysed with an extension of the ordinary regression model, that is, Tobit regression analysis [22]. The Tobit calculations were performed with the TSP program [23]. Logistic regression analysis has also been used.

## RESULTS

### *Anti-N. meningitidis serogroup A antibodies*

*Differences in seroprevalence between non-vaccinated Sudanese and Swedish children.* Anti-MC serogroup A antibodies of any immunoglobulin class (IgG, IgA or IgM) were more prevalent in the sera from non-vaccinated Sudanese children than from Swedish children ( $P = 0.035$ , chi-square, 3 df) (Table 1).

The cumulative frequencies of anti-MC serogroup A antibodies of IgG for different age groups from the two nations are shown in Fig. 1. Similar patterns were seen for IgA and IgM (not shown). Statistical modelling of the antibody levels, using Tobit regression analysis with Sudan as the reference, showed a significant decrease in IgG for Sweden,  $-122.3$  (s.e. 44.7,  $P < 0.05$ ), but not for IgA and IgM. In this first model, the age parameters were assumed to be equal for Sudan and Sweden and none reached statistical significance. In the succeeding models the age parameters were studied for Sudan *v.* Sweden (Table 2). Swedish children 1–4 years of age were found to have significantly lower IgG and IgA levels than the reference (Sudanese children 1–4 years) and Sudanese children of the oldest age group, 10–14 years, had significantly lower levels of IgA than the reference. Models where sex was included as an explanatory variable did not improve the model fitting. Alternative modelling using logistic regression analysis with the dependent variable defined as not detected/detected level of any antibody showed similar results.

*Differences between MC serogroup A vaccinated and non-vaccinated Sudanese children.* Anti MC serogroup A polysaccharide antibodies of any immunoglobulin class (IgG, IgA or IgM) were more prevalent in the sera from vaccinated ( $n = 34$ ) than from non-vaccinated ( $n = 168$ ) children in the age group 1–14 years. Analysing the difference in antibody levels with Tobit regression, the parameter estimate for vaccination was significantly different from zero for IgG ( $P < 0.01$ ) but not for IgA and IgM. An analysis with logistic regression aimed at not detected/detected IgA also showed significance ( $P < 0.01$ ).

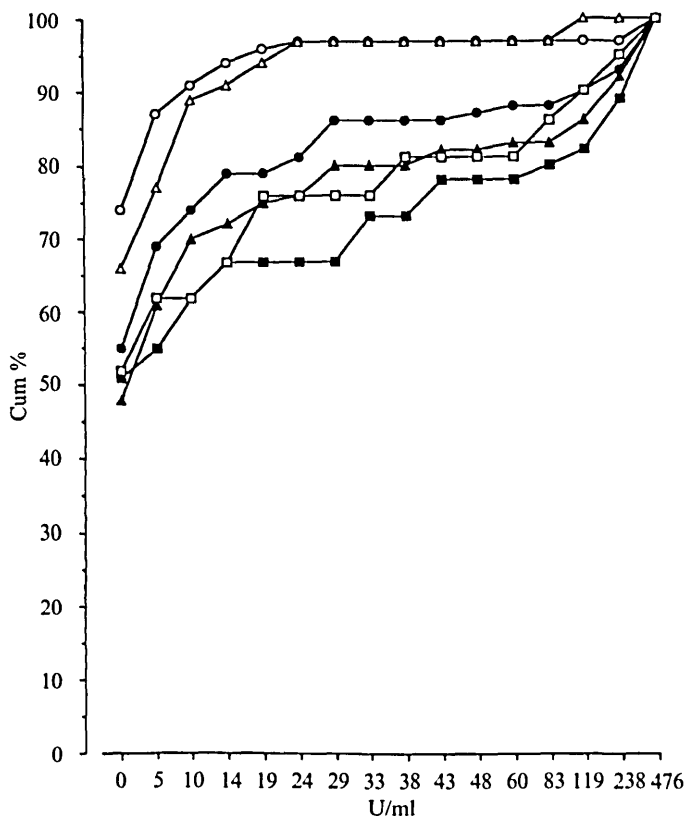


Fig. 1. Cumulative frequencies of anti-*N. meningitidis* serogroup A polysaccharide antibodies of IgG in three non-vaccinated age groups of Sudanese and Swedish children. Sixteen levels of antibody concentrations up to the level of the positive control (476 U/ml) are shown on the abscissa (not linear). Each point on a curve represents the proportion (%) of the population with antibody concentration up to the set level. Sudanese children 1-4 (●, *n* = 68), 5-9 (▲, *n* = 35), 10-14 (■, *n* = 21) years old. Swedish children 1-4 (○, *n* = 42), 5-9 (△, *n* = 71), 10-14 (□, *n* = 55) years old.

Table 2. *Adjusted\* mean deviations in IgG, IgA and IgM anti-N. meningitidis serogroup A polysaccharide antibodies in sera from non-vaccinated Sudanese and Swedish children. The deviations are expressed relative to the chosen reference group and with standard errors in brackets*

	Age group		
	1-4 years	5-9 years	10-14 years
Sweden <i>n</i>	42	71	55
Sudan <i>n</i>	68	35	21
IgG, Sudan	(reference)†	-40.2 (69.9)	6.7 (73.6)
IgG, Sweden	-140.6‡ (70.5)	-156.7 (82.2)	-93.1 (96.0)
IgA, Sudan	(reference)	-158.4 (85.1)	-185.4‡ (89.6)
IgA, Sweden	-168.9‡ (85.8)	-182.6 (100.1)	-193.1 (116.8)
IgM, Sudan	(reference)	-29.3 (168.3)	-302.9 (177.9)
IgM, Sweden	-296.2 (169.6)	-168.6 (197.8)	-91.9 (231.0)

\* Adjustment by Tobit regression models, see text.

† Sudan, 1-4 years, is a reference group also for the Swedish group.

‡ Different from zero at the 5% significance level or lower.

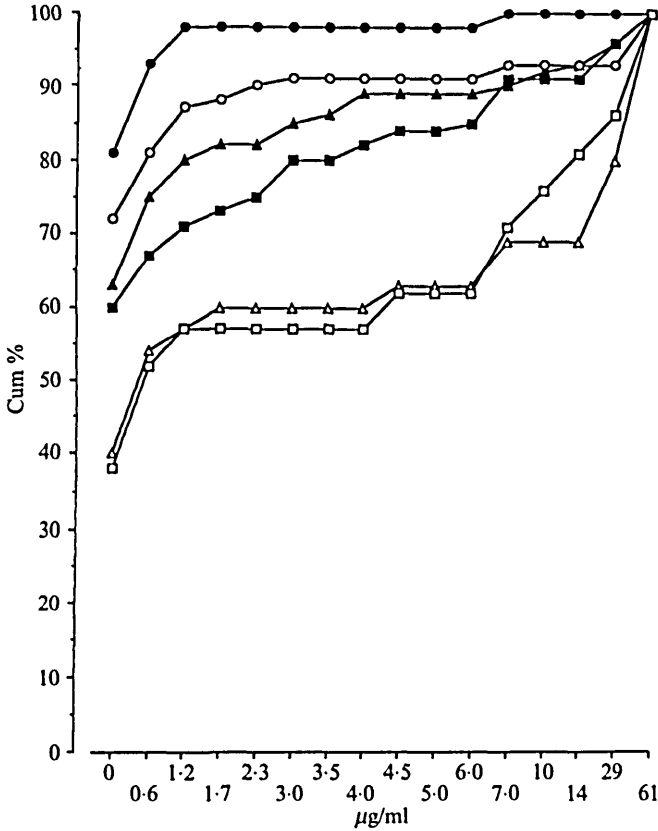


Fig. 2. Cumulative frequencies of anti-*H. influenzae* type b polysaccharide antibodies of IgG in three non-vaccinated age groups of Sudanese and Swedish children. Sixteen levels of antibody concentrations up to the set level of the positive control (61 µg/ml) are shown on the abscissa (not linear). Each point on a curve represents the proportion (%) of the population with an antibody concentration up to the set level. Sudanese children 1-4 (●,  $n = 68$ ), 5-9 (▲,  $n = 35$ ), 10-14 (■,  $n = 21$ ) years old. Swedish children 1-4 (○,  $n = 42$ ), 5-9 (△,  $n = 71$ ), 10-14 (□,  $n = 55$ ) years old.

#### *Anti-H. influenzae type b antibodies*

*Differences in seroprevalence between non-vaccinated Sudanese and Swedish children.* Anti-Hib antibodies of any immunoglobulin class (IgG, IgA and IgM) were more prevalent in sera from Swedish children than from the Sudanese ( $P = 0.002$ , chi-square, 3 df) (Table 1).

The cumulative frequencies of anti-Hib antibodies of IgG for different age groups from the two nations are shown in Fig. 2. Similar patterns were seen for IgA and IgM (not shown). Statistical modelling using Tobit regression analysis with Sudan as the reference showed a significant increase for Sweden for IgG, 146.0 (s.e. 35.4,  $P < 0.05$ ), as well as for IgA, 181.3 (s.e. 46.2,  $P < 0.05$ ), and IgM, 70.6 (s.e. 28.4,  $P < 0.05$ ). In this first model the age parameters were assumed to be equal for Sudan and Sweden. In the succeeding models the age parameters were studied for Sudan *v.* Sweden (Table 3). The main difference between Sudan and Sweden was shown to be in the age group 5-9 years. Here all parameters in the Swedish material showed significantly higher levels of antibodies than the

Table 3. *Adjusted\* mean deviations in IgG, IgA and IgM anti-H. influenzae type b polysaccharide antibodies in sera from non-vaccinated Sudanese and Swedish children. The deviations are expressed relative to the chosen reference group and with standard errors in brackets*

	Age group		
	1-4 years	5-9 years	10-14 years
Sweden <i>n</i>	42	71	55
Sudan <i>n</i>	68	35	21
IgG, Sudan	(reference)†	47.8 (54.8)	67.2 (57.7)
IgG, Sweden	66.1 (55.3)	316.1‡ (64.4)	161.2‡ (75.2)
IgA, Sudan	(reference)	19.7 (70.8)	33.4 (74.5)
IgA, Sweden	33.8 (71.3)	417.8‡ (83.2)	132.4 (97.1)
IgM, Sudan	(reference)	72.3 (44.0)	1.6 (46.4)
IgM, Sweden	-2.5 (44.4)	214.9‡ (51.8)	86.1 (60.5)

\* Adjustment by Tobit regression models, see text.

† Sudan, 1-4 years, is the reference group also for the Swedish group.

‡ Different from zero at the 5% level or lower.

Sudanese reference group. As in the previous analysis sex was not a significant variable. Alternative modelling using logistic regression analysis with the dependent variable defined as not detected/detected level of any antibody showed similar results.

## DISCUSSION

Several differences of antibody levels are shown in the two populations studied. Detectable serum antibody levels against MC serogroup A polysaccharide were significantly more frequently found in non-vaccinated Sudanese children than in Swedish children. However, the opposite finding was obtained with anti Hib-capsular antibodies which indicate that the ELISA results are reliable and not due to unspecific reactions in the tests with the African sera.

The Tobit regression model was chosen as the tool of analysis because of the specific statistical properties of the antibody levels. In case of detection of antibodies the levels can be treated as continuous variables well suited for regression models. However, at the specified point of detection the antibody levels must be censored, and all samples without detected levels must be given a constant and arbitrary value. Since zero detection was fairly common we will have a continuous effect variable with quite considerable censoring at the point of detection. This causes problems with the application of the ordinary regression model since the parameter estimates will be biased towards zero. Tobit regression is better suited to deal with this situation.

Furthermore the parameter estimates of the Tobit model can be interpreted in the same way as in the ordinary regression model. For example, in Table 2 the model for IgG with different age parameters for the two nations gives the estimate -156.7 for Swedish children in the age group 5-9 years. This value indicates that the antibody levels for this group of children on the average is 156.7 points lower than those of the reference group, the Sudanese children in the age group 1-4 years.

In our models shown in Tables 2 and 3, the relation between age and the antibody levels is not modelled linearly. Age is treated as a qualitative variable in three classes. The reason is the observed non-linear relation as indicated especially for Hib with the increased antibody levels for the middle age group 5–9 years but this was not found for MC of serogroup A.

Collection of sera from Sudanese children was conducted immediately before an epidemic of meningococcal meningitis due to a serogroup A MC strain which was new to the sub-Saharan meningitis belt [14]. In spite of that, the frequency of non-vaccinated children with detectable antibodies against MC serogroup A polysaccharide was significantly higher than in sera from Swedish children who did not experience any meningococcal epidemic. However, the same serogroup A meningococcal strain had been isolated in Sweden in 1987 [14], i.e. before serum sampling, but did not cause any epidemic. This fact indicates that other factors than herd immunity, as measured by serum antibodies against MC serogroup A polysaccharide, are important for avoidance of an epidemic due to a recently introduced serogroup A meningococcal strain. One factor may be antibodies directed against other surface-expressed components, e.g. outer membrane proteins of the meningococci. IgA antibodies blocking the bactericidal activity of serum have also been discussed [24]. Genetic and/or environmental risk factors such as climate may also be of importance [25]. It should, also, be noted that the age profile of the 1988 Sudanese epidemic was different compared with other countries [12] where the children were mostly affected. In the Sudanese epidemic the proportion of adults was greater and more than 50% were 15 years of age and older [25].

Anti MC serogroup A+C vaccines have, in several studies, been shown to be effective at preventing meningococcal disease [26–28]. With the present ELISA assay antibodies against MC serogroup A polysaccharide were detected at a significantly higher frequency in vaccinated than in non-vaccinated Sudanese children, which may, to some extent, explain why a lower proportion of the patients in the epidemic were children.

The incidence of invasive Hib infections in Sweden is high compared with other industrialized countries [29, 30] and Hib meningitis cases have increased in number in Sweden during recent decades [9]. Hib infections most often affect children [20, 29, 30]. Studies from different countries show that, in a population with a low socioeconomic standard, Hib infections are more frequent than in a population with a high socioeconomic standard [18, 19]. The present study of antibodies against Hib polysaccharide indicate that Swedish children were more frequently exposed to the Hib bacillus and/or micro-organisms with cross-reacting antigens than Sudanese children. It also shows that the frequency of Swedish children with antibodies was significantly greater in the group 5–9 years old than in both younger and older children. No such age-dependent difference was seen in Sudanese children. A possible explanation of the seroprevalence results may be the different systems of day-care of children in the two countries. In modern Sweden most of the preschool children from 1 year of age stay at day-care centres while most of the Sudanese children grow up in the extended family. This difference probably creates more diverse contacts between young children in Sweden than in Sudan and may explain the different seroprevalence pattern for



Hib. A prospective study of Hib disease is presently being conducted in Sweden in order to explore the impact of socioeconomic factors in this society.

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