Protective Effect of Breastfeeding on Invasive *Haemophilus influenzae* Infection: A Case-Control Study in Swedish Preschool Children

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**Background.** In Örebro County a 2.5-fold increase in the incidence of *Haemophilus influenzae* (HI) meningitis was found between 1970 and 1980, an observation that initiated the present study.

**Materials and methods.** In order to search for associations between morbidity in invasive HI infection and possible risk factors, a case-control study was conducted over a 6-year period from 1987 to 1992, before general Hib vaccination was introduced in Sweden. Fifty-four cases with invasive HI infection and 139 matched controls were studied for possible risk factors such as day-care outside the home, short duration of breastfeeding, passive smoking, low socioeconomic level of the household, many siblings in the family, allergy, frequent infections, repeated antibiotic treatments and immunoglobulin deficiency.

**Results.** Multivariate analysis showed a significant association between invasive HI infection and two independent factors, i.e. short duration (<13 weeks) of exclusive breastfeeding, odds ratio (OR) 3.79 (95% confidence interval [CI] 1.6–8.8) and history of frequent infections, OR 4.49 (95% CI : 1.0–21.0). For the age at onset 12 months or older, the associations were stronger, OR 7.79 (95% CI : 2.4–26.6) and 5.86 (95% CI : 1.1–30.6), respectively. When breastfeeding duration in weeks was analysed as a continuous variable the OR was 0.95 (95% CI : 0.92–0.99 ), indicating a decreased risk with each additional week. Increased OR were observed for other risk factors as well but not of the magnitude found for short duration of breastfeeding.

**Discussion.** The association of decreased risk for invasive HI infection and long duration of breastfeeding was persisting beyond the period of breastfeeding itself. This finding supports the hypothesis of a long-lasting protective effect of breastfeeding on the risk for invasive HI infection.

**Conclusion.** A decreased risk for invasive HI infection with long duration of breastfeeding was found. Our results do have implications for strategies in breastfeeding promotion, especially in countries where Hib vaccination is too costly and not yet implemented.

**Keywords:** breastfeeding, *Haemophilus influenzae* infection, case-control

Invasive *Haemophilus influenzae* (HI) infection is still an important cause of worldwide morbidity and mortality in childhood. Since the introduction of general vaccination against *Haemophilus influenzae* type b (Hib) in some areas, a decrease of HI incidence has been documented.1,2 Most of the invasive HI infections are due to type b (Hib) which may cause secondary cases in day-care centres and households.3–5 In populations such as eskimos and navajo indians with a very high incidence of invasive HI infection, meningitis is the most common form with a peak incidence below the age of one year6,7 while epiglottitis is seen in populations with a low incidence and with a peak around the age of 3 years.8,9 One of the most important risk factors for disease is subsequently young age. Other risk factors have been mentioned such as day-care attendance,10–13 passive smoking,1,13,14 household crowding,10–12 presence of siblings younger than 7 years of age, history of previous hospitalization or otitis media and breastfeeding for less than 6 months.15

In Sweden, Salwén et al. reported a 2.5 fold increase in incidence of HI meningitis between 1970 and 198016
and a high incidence has since then up to the introduction of a general Hib vaccination in 1993 persisted in a non-vaccinated Swedish population. The increased incidence of HI meningitis in Swedish children 1970–1980 coincided with the extension of the use of day-care centres, an observation that initiated the present study. In order to search for associations between morbidity in invasive HI infection and possible risk factors, a case-control study was designed and conducted over a 6-year period 1987–1992, before general Hib vaccination was introduced in Sweden. We hypothesized that there might be a positive association between morbidity in invasive HI infection and day care outside the home, short duration of breastfeeding, passive smoking, low socioeconomic level of the household, many siblings in the family, history of morbidity in allergy or infectious disease, repeated antibiotic treatments and immunoglobulin deficiency.

MATERIALS AND METHODS

Study Design
A prospective case-control study was conducted between 1 January 1987 and 31 December 1992 in Örebro County, Sweden, with a mean population of 271 490 during the study period. The population was ethnically homogenous. All cases of invasive HI infection among preschool children (<6 years of age) residing within the county were registered and studied by a team of physicians, a nurse and a statistician. The age-specific incidence of HI infection in the catchment area for the most affected age group, 0–4 years, was 55 per 100 000 person-years. For each case, three controls were selected from the child health centre (CHC) of the patient by the local nurse who chose children closest in age and of the same sex as the case. Thus the controls were matched for area of residency (same CHC), time (date of onset of invasive HI disease), sex and age.

Definitions
The diagnosis was based on clinical findings consistent with invasive HI infection with a positive culture from a normally sterile location (blood, cerebrospinal fluid, bone or joint fluid). The age for both the cases and controls was registered in months and calculated for the day of onset of the disease. The duration of breastfeeding was registered in weeks. By the term ‘exclusive’ breastfeeding we mean breastfeeding alone while ‘any’ breastfeeding means breastmilk alone or together with formula or solids. Day-care was defined as a regular supervised care of a child which took place at home, in another family home or in a day-care centre. A family member was defined as a person staying 14 nights per month or more in the family. Passive smoking was defined as a parent or caretaker smoking in the home or other family home, regardless if he/she was smoking inside or outside the room where the child was staying. Socioeconomic level was classified by the educational level of the head of the household by using a structural class measure used by Statistics Sweden. Allergy was defined as stated history of atopic symptoms such as eczema, seasonal rhinoconjunctivitis and/or obstructive bronchitis in a study person. History of frequent infections was recorded as stated by the parents.

Methods of Data Collection
After parental consent was obtained, the cases and the controls were investigated by the same method. Personal interviews were performed with the parents, usually the mother, using a questionnaire coding for previous state of health, family characteristics, type of day-care, passive smoking and breastfeeding pattern. Blood specimens were taken. The interviews were done and the samples taken at the ward during the treatment period for the cases and at home visits or at an outpatient clinic for the controls. For some single cases and controls only telephone interviews were done.

Ethics
The study was approved by the Örebro County Council’s Ethical Committee.

Laboratory Methods
Laboratory analyses were performed according to the routines of the Department of Clinical Microbiology and Immunology. Blood cultures were performed with the Bactec™ system which was also used for punctured material after addition of Fieldes supplement. Chocolate agar was used for culture on solid media and HI specificity ascertained by typical colony appearance, XV positivity, and if doubtful, with the rapid NH api. Typing was performed with commercial co-agglutination reagents.

Serum from the cases was collected in the late convalescent phase of the infection for assays of immunoglobulin classes (Ig) and IgG subclasses, using Mancini’s immunodiffusion technique. The reference intervals of Stiehm and Fudenberg and of Oxelius were used. All subnormal values were reassessed.

Statistics
Data from the interviews and the laboratory tests were continuously registered but no statistical analysis was performed before the end of the study when the data collection was completed. In a first univariate analysis cases were compared with controls for each risk factor.
Then a multivariate analysis was done where risk factors selected both with regard to the results from the univariate analysis and to the *a priori* hypotheses were used. Additional analyses were done with stratifications based on age at the occurrence of disease and of type of disease. The main analyses were done with conditional logistic regression and the results reported as odds ratios (OR) with 95% confidence intervals (95% CI). Interaction effects were tested and improvement in goodness-of-fit was judged by likelihood ratio testing. Supplementary analyses were done with the Mantel-Haenszel statistic in the univariate part, and with unconditional logistic regression in the multivariate part. In the latter analysis different modellings of the age effect were explored. Kaplan-Meier techniques displayed the breastfeeding duration for cases and controls.

**RESULTS**

Fifty-seven children met the criteria for inclusion as cases, 56 were due to type b and one to type f. Three cases and 23 invited controls were excluded due to unwillingness to participate. The remaining 54 cases together with 139 controls formed the study population. Of the 54 cases there were 29 with meningitis, 16 with epiglottitis and nine with other invasive HI infections. The mean age for cases was 21.6 months; 15.5 months for the meningitis cases and 31.5 months for the epiglottitis cases. The mean age for controls when interviewed was 23.6 months. Among the meningitis and the other invasive cases there was an equal distribution of boys and girls but for the epiglottitis cases there was a predominance of boys, 12 out of 16.

The breastfeeding durations were plotted in Kaplan-Meier diagrams (Figures 1 and 2). Based on the information from these diagrams dichotomizations were done at those points where the diagrams exhibited the maximum difference in breastfeeding proportion between the cases and the controls. Short duration of exclusive breastfeeding was subsequently defined as 0–12 weeks and long duration as 13 weeks or more. Short duration of any breastfeeding was defined as 0–20 weeks and long duration as 21 weeks or more. In the continued analysis breastfeeding pattern was analysed with either the continuous or the dichotomous representation.

In the univariate analysis, with the conditional logistic regression model, it was found that the duration of breastfeeding was associated with invasive HI infection (Table 1). Supplementary analysis with the Mantel-Haenszel technique gave similar results. In the case group, the mean duration of exclusive breastfeeding was 11.1 weeks and of any breastfeeding 17.6 weeks, while in the control group it was 14.5 and 22.7 weeks, respectively. When breastfeeding duration in weeks was analysed as a continuous variable it was found that the odds ratio (OR) was 0.95 (95% CI : 0.92–0.99), indicating a decreased risk with each additional week. For any breastfeeding the OR was 0.97 (95% CI : 0.94–0.99).

The multivariate analysis was performed with the duration of exclusive breastfeeding as a dichotomized variable (Table 2). Stratifications were done with age at onset of disease and with type of disease. The OR for exclusive breastfeeding duration was three or higher in all analyses. Type of disease did not change the estimates for the effect of breastfeeding to any appreciable
degree. Age at onset of disease did show interesting results. When children below 12 months of age with eventual ongoing breastfeeding, which theoretically could affect the estimation of the OR, were excluded, the analysis showed higher values of the OR.

The duration of exclusive and any breastfeeding was strongly correlated, $r = 0.74$. In this study group 161 out of 189 showed agreements of short and long duration of exclusive and any breastfeeding (79 short/short and 82 long/long). Six mothers had short exclusive
breastfeeding and long any breastfeeding, while 22 had long exclusive breastfeeding and short any breastfeeding. In an additional multivariate analysis, with long duration of both exclusive and any breastfeeding as reference category, long duration of exclusive and short duration of any breastfeeding showed an OR of 1.8, while short duration of exclusive and long duration of any breastfeeding had an OR of 3.5 and short duration of both exclusive and any breastfeeding had an OR of 4.4. It seems thus that long duration of exclusive breastfeeding is more beneficial than long duration of any breastfeeding.

In additional multivariate analyses some of the remaining explanatory variables were aggregated into fewer categories in search for a more parsimonious model. Breastfeeding showed OR significantly different from 1.0 in all these analyses. In comparison with the univariate analysis the estimates deviated further from unity. Breastfeeding was also analysed with the continuous representation and with any breastfeeding. In the multivariate model of Table 2 with continuous exclusive breastfeeding the OR was estimated at 0.95 (95% CI: 0.91–0.99). The effect of the stratification was similar, the OR deviated further from unity in the same way as for the dichotomous case. Any breastfeeding (dichotomous) applied in the same model gave an OR of 2.92 (95% CI: 1.3–6.5). The OR for any breastfeeding were generally somewhat lower than those for exclusive breastfeeding.

There were some indications that the conditional logistic regression was sensitive to sampling fluctuations in the smaller data sets of the stratified analyses. The unconditional logistic regression, with age at onset of disease and gender as added explanatory variables, was therefore used as a supplementary method of analysis. In general the unconditional logistic regression gave OR 10–15% closer to unity than the conditional method, but the results were otherwise consistent with the findings of the previous method.

Besides short duration of breastfeeding and history of repeated infections, no other potential risk factor showed significant evidence of being associated with an

<table>
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<th>Risk factor</th>
<th>Age at onset</th>
<th>Diagnosis</th>
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<tr>
<td></td>
<td>All</td>
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<td></td>
<td>OR CI</td>
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<td>2.02 0.6–6.7</td>
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<td>Frequent infections</td>
<td>4.49 1.0–21.0</td>
<td>5.86 1.1–30.6</td>
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increased risk of invasive HI infection. Estimates of increased OR were found for allergy, for siblings in the family and for attendance at a day-care centre but the confidence intervals were wide and the OR were not significantly different from unity.

We found a difference between the genders, among controls but not among cases, for mean breastfeeding duration. The mean values for exclusive breastfeeding in the case-group was 11.1 weeks for boys and 11.0 for girls and in the control group it was 13.3 and 16.1 weeks, respectively.

There were no significant differences between the cases and the controls concerning the geometric mean of each fraction of immunoglobulin, IgG, IgA, IgM or IgG subclasses, and specific IgE antibodies (Phadiatop, Pharmacia).

DISCUSSION
We found support for a hypothesis of a long-lasting protective effect of breastfeeding on invasive HI infection. When studied as a continuous variable, breastfeeding gave an overall long-term decreased risk of 0.95 for each week of breastfeeding, a finding which indicates a dose-response relationship.

We consider these results as valid for many reasons. Our study population is homogenous and there were no HI epidemics during the study period. The incidence of invasive HI infection is about the same in our population as reported in earlier publications involving Caucasians. For risk factors like short duration of breastfeeding, there is a good precision with narrow CI despite the relatively small size of the study population. Swedish children attend CHC, consequently all our patients with invasive HI infection were registered at the CHC and the matched control children could easily be identified. No serious classification problems should exist for duration of breastfeeding because it has been registered in weeks in order to maintain precision. The focus has been on breastfeeding duration and not on ongoing breastfeeding. The duration of breastfeeding was measured from the interview with the mother. Our clinical experience is that this information is valid and recall bias is not a problem for Swedish mothers. However, in a case-control study of an eskimo population it was suggested that the reliability of reports of breastfeeding after 6 months of age and during the time of weaning was questionable. In a random sample of 15 cases and 42 controls we performed a separate validation study of the breastfeeding data. Here we observed 77% agreement between the breastfeeding data in the questionnaire and the child health record for cases, and 72% agreement for controls. The cases tended to overestimate the duration of breastfeeding compared with health records while the inverse was found for controls. Thus should a bias in the estimation of the protective effect be present it is more likely to be an underestimation.

A potential risk for selection bias was inherent in the design of the study, i.e. the matching for CHC assumed that the centres had different breastfeeding policies. However, a large variability in breastfeeding patterns was found, both within and between CHC, and in combination with the chosen method of analysis—conditional logistic regression with matched sets—we do not expect differences in breastfeeding policies to effect our findings.

Children invited to be controls but who did not participate have not been studied further. Differential non-participation could potentially cause a bias. However, the non-participation rate was low—14% among controls and 5% among cases.

The direct protective effect of ongoing breastfeeding on invasive HI infections as well as other infections and diseases has been described before. Many studies the registration of breastfeeding duration has not been optimal. The questions have been focused on ongoing breastfeeding and without including periods of exclusive and any breastfeeding, factors that are important for the documentation of long-lasting effects. When the duration has been measured in months instead of in weeks, there is a risk of misinterpration and false negative results. A long-lasting effect of breastfeeding has been reported for invasive Hib infection, Hib meningitis, otitis media, gastrointestinal infection, Crohn’s disease and appendicitis. In a Finnish study, breastfeeding for more than 6 months was found to be protective for invasive Hib infection, but in another Finnish study it was found not to be protective for invasive pneumococcal infection.

Our finding that the association between long duration of breastfeeding and decreased risk for invasive HI infection is stronger in older children, when children presumably are more exposed to different micro-organisms than at younger ages, may be due to a long-lasting effect of breastfeeding which becomes evident after a certain latency period when a direct protective effect of breastfeeding has ceased. The long-lasting effect of breastfeeding may be due to a number of different mechanisms e.g. promotion of mucosal maturation by the presence of epidermal growth factors in breastmilk, via a network of idiotype antibodies in breastmilk and the development of anti-idiotype antibodies in the child, or via the special bacterial flora associated with breastfeeding that may give a favourable
basic immunity by, for example, cross-reacting antibodies.17,36–44
Reports from other fields underline a long-lasting protective effect of breastfeeding which also support an immunological mechanism. For example, the prevalence of diabetes mellitus is less frequent among those who have been breastfed,44 transplantations are favoured if breastfeeding has been given during infancy,45 in children who have been vaccinated against polio or Hib, the antibody response has been stronger and faster among those who have been breastfed than those who have not.46
Our findings of elevated OR for allergy (although not statistically significant) and history of frequent infections were in accordance with our hypothesis. Morbidity in otitis media has earlier been reported as a risk factor.15 In other studies factors such as passive smoking, household crowding and day care outside the home have been associated with increased risk for invasive HI infection.10–13 Our study may have been too small to verify these possible associations. Staying at home, passive smoking, short duration of breastfeeding and low socioeconomic level are in our study population strongly correlated variables. With a more diversified questionnaire and a larger study population some of these factors may have shown a significant association with invasive HI infection. There could be a bias in the selection of controls if children from families with psychosocial problems and low socioeconomic level were excluded by the local nurse or because parents were not interested in participating. We have not found any significant associations between low socioeconomic level and increased risk. The matching for residency (CHC) may partly have controlled for socioeconomic level, thus this possible risk may have been underestimated.
In conclusion, our study supports a long-lasting protective effect of breastfeeding on the risk for invasive HI infection. The protective effect of breastfeeding is already evident after 13 weeks of exclusive breastfeeding or 21 weeks of any breastfeeding and was found to last for months and years. Our results do have implications on strategies in breastfeeding promotion, especially in countries where Hib vaccination is too costly and not yet general. In order to understand further the long-lasting effect of breastfeeding on the risk of invasive HI infection further studies are needed.

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REFERENCES


