Neonatal Haemophilus influenzae infections

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Abstract
Nine cases of neonatal Haemophilus influenzae septicaemia were recorded in Finland during 1985–9; incidence was 2.8/100 000 live births, and 1-6% of all cases of neonatal septicaemia. The onset of the disease was early in all cases, ranging from 0–6 hours after delivery. Seven of the infants were preterm and three died (overall mortality 33%). H influenzae was isolated from blood in seven of the cases, and in two neonates with clinical signs of septicaemia it was found on several surface sites and the placenta. One of the eight strains of H influenzae was capsular type b and biotype I, the rest being nontypable—a distribution similar to those previously reported. Four of the uncapsulated strains were of biotype III, and three were of biotype II. None of the strains of H influenzae was of biotype IV, which has been reported to be characteristic of neonatal and genital isolates of H influenzae. All nine mothers had some sign of infection at the time of or shortly after delivery. H influenzae was isolated from the mothers: from the blood (n=1) or from the placenta or cervix (n=4).

The use of intrauterine devices may be a possible risk factor for neonatal H influenzae infections; two of the mothers had such devices in place during their pregnancies.

Neonatal Haemophilus influenzae infections were once thought to be rare, but during the past decades an apparent increase of such infections has been reported. Two H influenzae infections have been found mainly among preterm, low birthweight infants, and are characterised by the early onset of symptoms and the fulminant course of the disease. It has been suggested that these infections may have started during pregnancy and are the cause of the premature birth. The mortality associated with neonatal H influenzae septicaemia is high, up to 86% having been reported.

Most (80–90%) of the strains of H influenzae that cause neonatal septicaemia are nonencapsulated, whereas 95% of strains that cause invasive disease among older children are capsular type b. The neonatal strains and the strains that colonise the female genital tract have been reported to be predominantly biotype IV, and seldom biotype II or III, whereas most of strains with the type b capsule are of biotypes I or II.

A nationwide intensified surveillance of invasive infections in children (including neonates) was started in Finland in 1985 as a part of a trial of the efficacy of a vaccine against H influenzae capsular type b. We report the results of a five year prospective follow up of neonatal H influenzae infections in Finland and describe the clinical and bacteriological features of nine cases of neonatal H influenzae infection.

Patients and methods
In the nationwide surveillance all bacterial isolates from blood, cerebrospinal fluid, or other sterile body sites of children (0 to 15 years of age) with invasive disease were sent to the National Public Health Institute from the 25 microbiological laboratories in Finland. In addition, if a neonate (defined as an infant less than 28 days of age) was thought clinically to have septicaemia and the same bacterial species was isolated from several surface sites (for example, ear, umbilicus, or throat) and the placenta, this strain was included in the collection. The identification of strains was confirmed at the National Public Health Institute and samples were stored at −70°C. The laboratory records from the years 1985–9 in each of the laboratories were also examined retrospectively to find possible omissions from the collection. Detailed data about the patients and their mothers were collected from the hospital records.

The capsular types (a–f) of the strains of H influenzae were ascertained by a coagglutination technique. Strains were designated to biotypes I–VIII on the basis of their urease and ornithine decarboxylase activity and the production of indole. Production of β-lactamase was tested by the clover leaf method or the acido- metric assay, or both.

Results
CLINICAL FEATURES AMONG THE NEONATES
During the five year study period from January 1985 to December 1989, nine neonates with H influenzae septicaemia were recorded. During that time there were 317 673 live births in Finland, giving an annual incidence of 2-8/100 000 live births. All nine cases were identified by the prospective surveillance, and no further cases were found through the retrospective evaluation of laboratory records. During the same period the surveillance yielded a total of 547 cases of invasive neonatal disease, with positive bacterial cultures from blood, cerebrospinal fluid, or any other usually sterile body fluid. H influenzae comprised 16% of all neonatal infections. In seven neonates H influenzae was isolated from the blood and in two cases from several surface sites as well as from the placenta. None
of the nine cases had signs of meningitis, and the cerebrospinal fluid was not cultured from any of the patients.

The onset of disease was early in all the cases, ranging from 0–6 hours after delivery (table 1). Seven patients were preterm (gestational age range 25–35 weeks) and weighed less than 2500 g at birth. All three deaths occurred among the preterm neonates.

*Home delivery.

H influenzae septicemia was accompanied by raised (>30×10⁹/l) white cell count in two, by leucopenia (<8×10⁹/l) in three, and by normal values in four of the patients. All six neonates from whom serum C reactive protein concentrations were available had increased values (≥20 mg/l). Respiratory distress syndrome was noted in five neonates, all of whom were preterm (gestational age 25–30 weeks) (table 1).

Only one child had septicemia caused by type b H influenzae (case 3, table 1). This was the mother’s third pregnancy and delivery. She had an intrauterine device in place and was not aware of the pregnancy until the delivery started at 30 weeks’ gestation. The child was born at home with a nurse in attendance. Immediately after birth (the labour lasted 1.5 hours) the child was transferred to hospital. On arrival he had breathing difficulties with hyperventilation, bradycardia, hypotonia, and hypothermia. After intubation, and correction of his haemodynamic state, his condition improved. After blood had been taken for culture, antimicrobial treatment with penicillin G and netilmicin was started. Mild respiratory distress syndrome developed and he required two days on the ventilator. Antibiotic treatment was stopped on day 3, before the results of the blood culture were available. As no further signs of septicemia developed, and the white cell count and C reactive protein remained normal, however, the antibiotic treatment was not started again. The child recovered uneventfully and his development has been normal.

ISOLATES OF H INFLUENZAE FROM NEONATES
Isolates of H influenzae were available from eight neonates (table 1). In only the above-mentioned case was the isolate of capsular type b. It was of biotype I and produced β-lactamase. The rest of the isolates were non-typable. Four of these were biotype III and three biotype II. None of the non-typable isolates produced β-lactamase. The antimicrobial treatment given was according to the regimens in the respective hospitals, and in most cases was a combination of netilmicin and ampicillin.

CLINICAL FEATURES AND ISOLATES OF H INFLUENZAE AMONG THE MOTHERS
Seven of the nine mothers had signs of infection before or during delivery (table 2), and the remaining two (cases 1 and 4) had raised white cell counts or C reactive protein concentrations, or both. In five cases H influenzae was isolated from blood, from the placenta, or from the cervix. Unfortunately the strains were not preserved for typing. In three cases there was premature rupture of the membranes. The role of

<table>
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<th>Case No</th>
<th>Sex</th>
<th>Gestation (weeks)</th>
<th>Birth weight (g)</th>
<th>Apgar score 1 min</th>
<th>Apgar score 5 min</th>
<th>Time from birth of symptoms (hours)</th>
<th>Outcome</th>
<th>H influenzae</th>
<th>Site of isolation</th>
<th>Capsular type</th>
<th>Biotype</th>
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<td>III</td>
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<tr>
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<td>Blood</td>
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*Home delivery.
intraterine devices emerged as a possible risk factor in neonatal *H influenzae* infections, as two of the mothers had devices in place during their pregnancies.

**Discussion**

In the United States between 2·6% and 7·9% of all neonatal septicaemias are caused by *H influenzae* and an apparent increase of these infections has been reported during the past decades. In addition, a report describing neonatal septicaemia in seven centres in Finland during a 10 year period covering roughly 43% of all deliveries in Finland was published in 1989. In that study, a total of six cases of *H influenzae* septicaemia were found, three during each five year period, 1976–80 and 1981–5.

The present study describes a nationwide study for the five year period 1985–9 in which nine cases of neonatal *H influenzae* infection were identified by intensified prospective surveillance. It seems therefore that all cases of neonatal *H influenzae* infection from that period are included. Compared with the previous report from Finland there seems to have been no increase in the incidence neonatal *H influenzae* infections during the past 15 years, and *H influenzae* infections account for only a minority, 1·6%, of all neonatal septicaemias.

Our study includes two cases in which *H influenzae* was not isolated from blood, but from several surface sites as well as from the placenta. Both these neonates had clincal signs of septicaemia, there was a raised white cell count (33·1×10^9/l) in one case and raised concentrations of C reactive protein in both cases (171 mg/l and 102 mg/l, respectively). The value of culturing a *H influenzae* from surface sites is supported by the study in which 70% of neonates with blood cultures growing *H influenzae* also had positive cultures from surface sites. All the six patients in whom C reactive protein concentrations were assayed had increased values. In contrast to a previous report in which it was suggested that the measurement of C reactive protein is no use in the diagnosis of neonatal septicaemia, our results suggest that in *H influenzae* septicaemia high concentrations of C reactive protein do occur, and the measurement of the concentration might be useful.

The clinical course of neonatal *H influenzae* infection was characterized by prematurity, the presence of maternal complications, and high neonatal mortality, as has been reported previously. In the present series all the cases developed early—that is, at delivery (seven cases) or within a few hours after delivery. This compares with earlier reports in which 85% of cases were of early onset defined as less than 24 hours after delivery.

All the mothers in the present study had some signs of infection at the time of, or shortly after, delivery and *H influenzae* was isolated from 56% of them. Premature rupture of membranes was not a serious predisposing factor to neonatal infection as previously suggested. These findings support the hypothesis that neonatal *H influenzae* infections are transmitted from the mother, that the infection starts before birth, and that it could be the cause of premature birth. Two of the mothers had interuterine devices in place, a possible predisposing factor for *H influenzae* infection. An association between intrauterine devices and neonatal *H influenzae* infection has previously not been reported, and there are only few case reports about other pathogens (*Escherichia coli* and *Candida albicans*) associated with neonatal septicaemia and intrauterine devices.

Seven of the eight neonatal isolates of *H influenzae* in the present series were non-typable, and there was one isolate of capsular type b, a finding similar to previous reports. The relative absence of type b isolates causing neonatal *H influenzae* infections is thought to be the result of maternal anti-*H influenzae* type b antibodies, which last for roughly the first six months of life; after this age the incidence of type b *H influenzae* infections increases appreciably. In addition, the colonisation of the female genital tract with type b *H influenzae* is rare: carriage of *H influenzae* is about 1%, and of the isolates only a few are type b. Preliminary results indicate that serum antibodies against non-typable *H influenzae* are relatively high among female population of child bearing age (M Leinonen, personal communication). This may partly explain the rarity of neonatal *H influenzae* infections despite the 1% vaginal carriage rate of non-typable *H influenzae*. None of the non-typable isolates of *H influenzae* were of biotype IV, which has been reported to account for up to 38% of neonatal isolates in the United States. Geographical differences have also been found in the comparison of isolates of type b *H influenzae* that cause invasive infections among older children, using several subtyping methods including biotyping. In these infections biotypes I and II both account
for about half the isolates in Finland, while biotype I predominates (90%) in the United States and The Netherlands. Geographical differences have also been found among strains of the most common neonatal pathogen, group B streptococcus, with serotype III accounting for a third of cases of early onset in the United States compared with only 10% in Finland. It has been suggested that there could be a difference in the virulence of these strains, leading to a predominance of group B streptococcal disease of early onset and a relative absence of disease of late onset in Finland, a similar difference to that found in the present study of neonatal H influenzae septicaemia. It might be that the emergence of biotype IV H influenzae as a genital pathogen in the United States is the cause for the increase of neonatal H influenzae infections.

In Finland these strains (or this clone) do not occur, and thus the incidence of neonatal H influenzae infections has been stable. This clone may also have special properties of virulence, as has been documented for H influenzae type b isolates of a certain clone characteristic to Finland.27