Neonatal Invasive Haemophilus influenzae Disease in England and Wales: Epidemiology, Clinical Characteristics, and Outcome

Sarah Collins,1 David J. Litt,2 Sally Flynn,2 Mary E. Ramsay,1 Mary P. E. Slack,2 and Shamez N. Ladhani1

1Immunisation, Hepatitis and Blood Safety Department, and 2Respiratory and Vaccine Preventable Bacterial Reference Unit, Public Health England, London, United Kingdom

Background. Nontypeable Haemophilus influenzae (NTHi) frequently causes noninvasive upper respiratory tract infections in children but can cause invasive disease, mainly in older adults. An increased burden of invasive NTHi disease in the perinatal period has been reported by a number of studies. Here we describe the epidemiology, clinical characteristics, and outcome of neonatal invasive H. influenzae disease in England and Wales over a 5-year period.


Results. Overall, 118 live-born neonates had laboratory-confirmed invasive H. influenzae disease: 115 (97%) were NTHi, 2 were serotype f, and 1 was serotype b. NTHi was isolated within 48 hours of birth (early-onset) in 110 of 115 (96%) cases, and 70 of 110 (64%) presented with septicemia. Only 17 mothers (15%) had suspected bacterial infection requiring antibiotics during labor. Few (8/110 [7%]) neonates had comorbidities. The incidence of early-onset NTHi increased exponentially with prematurity, from 0.9 per 100 000 (95% confidence interval [CI], .6–1.4) in term neonates to 342 per 100 000 (95% CI, 233.9–482.7) in neonates born at <28 weeks’ gestation (incidence rate ratio, 365 [95% CI, 205–659]; P < .001). Case fatality for early-onset NTHi was 19% (21/110); each additional gestational week reduced the odds of dying by 21% (odds ratio, 0.79 [95% CI, .69–.90]; P < .01). A quarter of neonates who survived experienced long-term complications.

Conclusions. Early-onset neonatal NTHi disease is strongly associated with premature birth and causes significant morbidity and mortality.

Keywords. Haemophilus influenzae; epidemiology; neonatal; outcome.

Haemophilus influenzae commonly colonizes the human upper respiratory tract and can be distinguished according to its polysaccharide capsule into 6 serotypes (a–f). Nonencapsulated or nontypeable H. influenzae (NTHi) lacks a polysaccharide capsule. Routine immunization against serotype b (Hib), previously a major cause of invasive H. influenzae disease, has resulted in a sustained decline in invasive Hib disease across all age groups through direct and indirect (herd) protection [1]. As a consequence, NTHi is now responsible for most invasive H. influenzae infections, mainly in older adults [2].

In children, NTHi usually causes noninvasive upper respiratory tract infections, such as otitis media and sinusitis [3]. Occasionally, although not as well-recognized as Hib, NTHi can also cause invasive disease, including pneumonia, septicemia, and meningitis, mainly in those with comorbidities [4]. Additionally, studies have identified a substantial burden of perinatal NTHi disease, causing significant morbidity and mortality in pregnant women and newborn infants [5]. In England and Wales, we estimated that the incidence of invasive
NTHi infection in pregnant women was >17 times higher than that in nonpregnant women [6]. Invasive NTHi disease in pregnant women is associated with serious illness, often resulting in septic abortion, intrauterine death, stillbirths, and premature birth [5–7]. In neonates, NTHi is 10-fold more common than Hib, with most cases presenting in the first week of life [2].

To better understand this uncommon but potentially fatal neonatal infection, we collected detailed clinical information on all laboratory-confirmed invasive neonatal H. influenzae infections in England and Wales. This study describes the epidemiology of neonatal invasive H. influenzae disease over a 14-year period (2000–2013), along with clinical presentation, comorbidities, complications, and outcome for cases diagnosed during 2009–2013.

METHODS

Public Health England (PHE) conducts enhanced national surveillance of invasive H. influenzae disease in England and Wales through a combination of clinical and laboratory reporting schemes and provides a national service for H. influenzae serotyping through its Haemophilus Reference Unit [1, 8]. PHE receives electronic laboratory reports of clinically significant invasive pathogens from National Health Service laboratories [9], which are then routinely contacted to submit the reported isolate to PHE if not already done [8, 10]. Clinicians, microbiologists, and public health doctors are also encouraged to report all invasive H. influenzae cases to PHE and to refer the isolate for species confirmation and serotyping [8]. Invasive H. influenzae disease was defined as isolation of the organism from a normally sterile site. Localized infections such as epiglottitis or pneumonia were included if accompanied by a sterile site isolate. Isolates were confirmed as H. influenzae by their growth requirement for X and V factors [11] and ompP2-specific polymerase chain reaction (PCR) positivity [12]. Capsulation status was determined using standard slide agglutination in conjunction with PCR detection of bexA and targets for each of the 6 capsule types [13, 14].

Cases of laboratory-confirmed invasive H. influenzae diagnosed in the first month of life (≤31 days) during 2009–2013 were followed up by sending a questionnaire to the patient’s general practitioner (GP) and requesting a copy of the patient’s hospital discharge summary. Additional follow-up of nonresponders and incomplete/inconsistent questionnaires was performed by letter and/or telephone to the patient’s GP and/or hospital clinician. Ethnicity was assigned by the clinician from the patient’s general practice or hospital record [15]. Post-mortem reports were obtained for all fatal cases from the coroner or histopathologist. In July 2014, the final outcome of all patients was confirmed and the cause of death obtained from the Office for National Statistics (ONS) death registration data provided to PHE for public health surveillance.

Data were analyzed using Stata software version 11.0 (StatCorp LP, College Station, Texas). Continuous variables that did not follow a normal distribution were described as median and interquartile range (IQR) and compared using the Mann–Whitney U test. Categorical variables were expressed as proportions and compared using the χ² test or Fisher exact test. ONS annual live-birth estimates were used to ascertain the denominator population. Where available, maternal characteristics were compared with national rates, including age, ethnicity, and type of delivery [16–18]. Incidence by gestation was calculated by applying the gestational stratification of births in England [16] to the total population in England and Wales during 2009–2013.

PHE has approval under the Health and Social Care Act 2001 to process confidential patient information for public health purposes (see http://www.legislation.hmso.gov.uk/si/si2002/20021438.htm).

RESULTS

Epidemiology

There were 366 neonatal invasive H. influenzae cases in England and Wales during 2000–2013. Of these, 81% (n = 296) were serotyped, including 281 (95%) NTHi, 10 (3%) Hib, and 5 (2%) serotype f (Hif). There were no cases of serotype a, c, d, or e in this age group. Adjusting for changes in proportion of isolates serotyped, the annual incidence of invasive NTHi disease ranged between 2.1 (95% confidence interval [CI], 1.7–2.5) and 4.8 (95% CI, 4.3–5.3) per 100,000 live births (Figure 1).

Figure 1. Adjusted annual incidence of invasive nontypeable Haemophilus influenzae disease in neonates in England and Wales, 2000–2013.
During the 5-year clinical follow-up (2009–2013), 124 sterile-site H. influenzae isolates were submitted to PHE for confirmation and serotyping. Surveillance questionnaires were returned for all 124 cases. Six isolates taken postmortem were excluded from further analysis. In 2 cases, the isolates were obtained from postmortem blood culture, but the final diagnosis was "unascertained" and "sudden infant death syndrome." The other 4 isolates were obtained from 3 stillbirths and 1 septic abortion of a fetus of 23 weeks’ gestation. In all 4 cases, there was postmortem evidence of maternal chorioamnionitis, with fulminant sepsis (including pneumonia) in the infants. The remaining isolates were obtained from 118 live births, including 115 NTHi, 1 Hib, and 2 Hif.

### Invasive NTHi Disease

Among 115 NTHi cases, 64 (56%) were male and 89% (n = 102) were white or white British. All but 5 infants (110/115 [96%]) had NTHi isolated within 48 hours of birth (early-onset infection), mainly on the day of birth (100/110 [87%]).

### Maternal Factors in Early-Onset NTHi Infections (n = 110)

Maternal characteristics were similar to national maternal statistics (Table 1), except that mothers of neonates with NTHi disease were more likely to be younger and primiparous; 19% were aged 16–19 years vs 5% nationally (z = 6.74, P < .01) and 53% were primiparous vs 42% nationally (z = 2.34, P = .02). Most women (98/110 [89%]) were white or white British (Table 1), with no association between ethnicity and maternal age (χ² = 22.97, P = .02), number of previous births (χ² = 8.91, P = .18), or presence of maternal comorbidities (χ² = 4.85, P = .18).

Seventeen women (15%) had suspected bacterial infection requiring intravenous antibiotics during labor, including...
pneumonia, genitourinary infection, and cholecystitis. Premature rupture of membranes (PROM) occurred in 35% (39/110) of cases (Table 1). There was no association between PROM and maternal parity ($\chi^2 = 18.77, P = .22$), maternal risk factors ($\chi^2 = 0.68, P = .88$), or the presence of maternal infection ($\chi^2 = 2.96, P = .40$). Labor occurred spontaneously in 85% of cases, with 65% of births (72/110) following spontaneous vaginal delivery and 26% (n = 29) by emergency cesarean delivery, mostly for fetal distress (24/29 [83%]).

**Early-Onset Invasive NTHi Disease**

Sixty-one infants with early-onset NTHi disease (53%) were male. Only 8 (7.3%), including 5 born prematurely, had comorbidities: congenital diaphragmatic hernia (n = 2), severe hypoxic-ischemic encephalopathy (n = 2), autosomal polycystic kidney disease (n = 1), congenital heart disease (n = 1), hypothyroidism (n = 1), and meconium aspiration syndrome (n = 1).

The overall incidence of early-onset invasive NTHi disease was 4.1 per 100 000 (95% CI, 3.4–5.0). Incidence was significantly higher (incidence rate ratio [IRR], 30.3 [95% CI, 18.8–50.8]; $P < .01$) in infants born prematurely (28.4/100 000 [95% CI, 22.8–35.0]) compared with those born at term (0.9/100 000 [95% CI, .6–1.4]) and increased exponentially with increasing prematurity (Figure 2). Infants born at <28 weeks’ gestation (342/100 000 [95% CI, 234–483]) were 365 times more likely to develop invasive NTHi disease compared with term infants (IRR, 365 [95% CI, 205–659]; $P < .001$).

Septicemia was the most common presentation (70/110 [64%]); 26% (29/110) had pneumonia, and 10% (11/110) had meningitis. Most infants (92/110 [84%]) were admitted to a neonatal intensive care unit for further management; the remaining infants were managed in the postnatal ward.

At last follow-up in July 2014, 21 infants had died (case fatality rate [CFR], 19%), and case fatality increased with prematurity (Table 2). Fifteen neonates died within 7 days of birth; 5 others died within 28 days, and 1 (born prematurely at <28 weeks’ gestation) died at 20 weeks of age from necrotizing enterocolitis. The CFR was higher among neonates with septicemia (16/70 [23%]) than for those with pneumonia (4/29 [14%]), and only 1 neonate with meningitis died (1/111 [9%]). The overall odds of an infant with early-onset NTHi infection dying decreased by 21% per additional gestational week (odds ratio, 0.79 [95% CI, .69–.90]; $P < .01$); the clinical presentation was not a significant factor ($\chi^2 = 2.842, P = .42$) (Table 2).

At discharge, a quarter of neonates who survived their infection had long-term complications resulting from a combination of being born prematurely and developing early-onset NTHi disease. Eleven of 54 neonates (20%) with septicemia developed complications, including chronic respiratory distress syndrome (n = 6), intraventricular hemorrhage (n = 3), cerebral palsy (n = 2), hydrocephalus (n = 1), seizures (n = 1), multiple liver abscesses (n = 1), acute renal failure (n = 1), and retinopathy of prematurity (n = 1). Six of 25 neonates (24%) who survived pneumonia had chronic respiratory distress syndrome; 1 also developed hydrocephalus, and another suffered from multiple pneumothoraces. Five of the 10 neonates (50%) who survived meningitis had significant long-term complications, including intraventricular hemorrhage, stroke, seizures, hydrocephalus requiring ventriculoperitoneal shunt insertion, and hemiparesis.

**Late-Onset Invasive H. influenzae Disease**

Five neonates developed invasive NTHi disease >48 hours after birth. One neonate born at term developed NTHi pneumonia

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**Table 2. Infant Factors in Early-Onset Invasive Nontypeable Haemophilus influenzae Infections (n = 110)**

<table>
<thead>
<tr>
<th>Factor</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>49 (44.5)</td>
</tr>
<tr>
<td>Male</td>
<td>61 (55.5)</td>
</tr>
<tr>
<td>Clinical presentation</td>
<td></td>
</tr>
<tr>
<td>Septicemia</td>
<td>70 (63.6)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>29 (26.4)</td>
</tr>
<tr>
<td>Meningitis</td>
<td>11 (10.0)</td>
</tr>
<tr>
<td>Outcome (died)</td>
<td></td>
</tr>
<tr>
<td>&lt;28 wk</td>
<td>12 (10.9)</td>
</tr>
<tr>
<td>28–31 wk</td>
<td>6 (5.5)</td>
</tr>
<tr>
<td>32–36 wk</td>
<td>2 (1.8)</td>
</tr>
<tr>
<td>Term</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td>Septicemia</td>
<td>16 (22.9)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>4 (13.8)</td>
</tr>
<tr>
<td>Meningitis</td>
<td>1 (9.1)</td>
</tr>
</tbody>
</table>

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secondary to influenza A(H1N1) infection in the fourth week of life and required intensive care support, but survived without complications. Another neonate born at 30 weeks’ gestation presented with pneumonia and developed multiple episodes of pneumothorax before recovering. The remaining 3 neonates had all been born prematurely and developed septicemia on days 3–9 of life; 2 survived and 1 died from complications of extreme prematurity.

The 3 late-onset cases of encapsulated H. influenzae infections occurred in previously healthy neonates. One of the mothers had invasive H. influenzae meningitis, and her infant died of H. influenzae meningitis in the first week of life. The other 2 mothers were not unwell during the peripartum period. One neonate presented with Hib meningitis in the second week of life and recovered without complications; the other developed Hib pneumonia secondary to respiratory syncytial virus infection in the fourth week of life and required invasive ventilation, but recovered without sequelae.

DISCUSSION

In England and Wales, the incidence of laboratory-confirmed neonatal invasive NTHi disease ranges between 2.1 and 4.8 cases per 100 000 live births, with nearly all infections occurring around the time of birth. Most infections were associated with premature birth; infants born before 28 weeks’ gestation had a significantly higher risk of invasive NTHi disease compared with term infants. In the vast majority of pregnancies, there were no other recorded maternal risk factors, and most women were well at the time of delivery. Compared with national statistics, the mothers of neonates with early-onset NTHi infection were younger and more likely to be primiparous.

In neonates, invasive NTHi disease was associated with significant case fatality and long-term morbidity among survivors, which often resulted from the NTHi infection as well as the well-known long-term complications of prematurity [19]. Moreover, there is increasing evidence that neonatal sepsis is in itself independently associated with adverse long-term neurodevelopmental outcomes among survivors [19].

In countries that routinely immunize against Hib, NTHi is responsible for nearly all invasive H. influenzae infections, mainly among older adults with comorbidities, and especially those with chronic obstructive pulmonary disease, who often present with pneumonia [4]. In addition, we and others have observed an increased burden of invasive NTHi disease in the perinatal period, but the literature is limited to case reports and small case series highlighting the severity of infection in the mother and newborn [5]. Notably, a Finnish study identified 7 early-onset cases during 1985–1989 (2.8/100 000 live births; 1.6% of neonatal septicemia cases), among which 5 infants were premature and 3 died [20]. Our estimated incidence for early-onset invasive NTHi disease is similar to invasive pneumococcal disease in the same age group in England and Wales (approximately 5/100 000 live births) [21], but substantially lower than that reported for group B streptococcal (GBS) disease (48/100 000 live births) [22], the single most common cause of neonatal septicemia and meningitis. Of note, our incidence was twice that reported by a UK study during 1996–1998 in which the annual estimated incidence was 1.6–1.9 per 100 000 for early-onset invasive NTHi disease [23].

The true burden of perinatal NTHi disease, however, is likely to be significantly higher than our estimates, which do not consider NTHi infections in pregnancy. During 2009–2012, we identified an additional 72 pregnant women with invasive NTHi infection [6]. Among these pregnancies, which included 2 twin births, 43 resulted in miscarriage, 2 were stillborn, and 29 were live born, of whom 11 were born prematurely. Of the survivors, nearly all required intensive care support for suspected sepsis but subsequently had negative cultures, most likely because their mothers had received antibiotics during labor, as per national recommendations [24]. Additionally, our estimated rates only include live births. In the United Kingdom, cases of miscarriage, septic abortions, and stillbirths rarely undergo postmortem examination, except in a few specialist centers. Even when H. influenzae is isolated postmortem, it is rarely considered a significant pathogen and, therefore, is not submitted for serotyping.

Overall, therefore, when considering the peripartum period to include the pregnant woman, the fetus, and the newborn, the burden of invasive NTHi disease is likely to be much higher than currently perceived and of greater importance because of the poor pregnancy outcomes. In keeping with this, active case finding in Tours, France, during 1979–1987 reported rates of Haemophilus species in mother–infant infections to be as high as 280 per 100 000 live births [25]. It is, therefore, vitally important that microbiology laboratories also culture bacterial specimens from pregnant women and neonates specifically for H. influenzae.

Our study also confirms previous studies reporting an association between neonatal NTHi disease, premature birth (approximately 75% of cases), and early-onset (<48 hours) infection [2, 23, 26]. In pregnant women, we observed invasive NTHi infections occurring across all trimesters and invariably resulting in induction of labor. Fetal outcome, therefore, was dependent on the pregnancy stage at which the mother developed invasive NTHi disease; infection was associated with miscarriage in first half of pregnancy and with premature birth and stillbirth in the second. Moreover, we have, for the first time, identified an exponentially increased risk of invasive NTHi disease with increasing prematurity, which has been also been observed with neonatal GBS disease [22].
The finding that nearly all the neonatal NTHi infections occurred around the time of birth is consistent with a US study [26] and an earlier UK study [23] where 92% and 85%, respectively, of neonatal cases were diagnosed within 7 days of birth. This, along with our previous observations that nearly all NTHi infections in pregnancy were associated with labor [6], suggests that the infection’s role in inducing labor could contribute to fetal distress, resulting in emergency cesarean section for some cases. Whether this is specific to NTHi or is a consequence of any serious infection during pregnancy is difficult to speculate [27, 28].

It is interesting to note that, unlike the pregnant women with invasive NTHi disease who were all symptomatic around the time of delivery [6], only 15% of the mothers in the current cohort had symptoms of infection, although more than a quarter underwent emergency cesarean delivery, mostly for fetal distress, and almost 40% had reported chorioamnionitis, with offensive or meconium-stained liquor at birth. It is possible that ascending infection from the lower genital tract may play a more important role than transplacental spread following bacteria in the mother. Unlike GBS, which commonly colonizes the female genital tract, NTHi genital carriage rates are low (1.8/1000 pregnant women) [29], although higher rates have been reported in women with PROM (8/110 [7.3%]) [30]. Although uncommon, NTHi has been associated with pelvic inflammatory disease in nonpregnant women [6, 31]. An emerging condition called aerobic vaginitis or desquamative inflammatory vaginitis has been linked to vaginal infection with respiratory or enteric pathogens; future studies should address whether NTHi vaginal infection is associated with inflammatory vaginitis, as this could aid clinicians to recognize potential women at risk [32, 33].

It has been proposed that the genital NTHi strains are different to those identified in the nasopharynx, with biotype IV strains recovered more frequently at the former site following disease [25]. Characterization of these biotype IV genital tract strains from mothers and neonates suggested that they may comprise a cryptic genospecies more adapted to the genital tract and capable of causing localized genital tract infections which, in pregnant women, could result in induction of labor and serious illness in the mother and newborn [25, 34]. Biotyping is not routinely performed on NTHi strains to PHE, but the accumulating evidence for NTHi as a significant cause of maternal and neonatal infections warrants more detailed characterization of the clinical isolates responsible.

Although this is by far the most comprehensive follow-up of neonates with invasive NTHi disease, our estimate of disease burden during this vulnerable period is likely to be significantly underestimated for reasons already discussed. Additionally, there will be some underreporting common to all national surveillance programs. The use of multiple electronic laboratory and clinical reporting systems and a requirement to report all cases of invasive \textit{H. influenzae} disease to PHE [9], along with a national reference laboratory service for serotyping that actively requests isolate submission for all reported cases and national public health guidance recommending antibiotic chemoprophylaxis for close contacts of confirmed Hib cases [35] (which requires serotyping of invasive isolates), ensures high case ascertainment across all age groups [6]. Our surveillance, however, does not include noninvasive isolates, such as those from the genital tract, or infections without an isolate, such as nonbacterial pneumonia, which may also cause a significant burden of illness in pregnant women, the fetus, and the newborn. The lack of mother–infant pairs with submitted isolates highlights the importance of considering both groups when assessing disease burden during this critical period. This could be facilitated by a single set of combined maternal/neonatal notes, which would be benefit clinicians, public health practitioners, and researchers.

Our study provides evidence of a significantly higher burden of invasive NTHi disease in the perinatal period than previously perceived, affecting the pregnant woman, the fetus, and the neonate. The high miscarriage rates following infection in early pregnancy, along with the exponentially increased risk of disease with increasing prematurity and identification of NTHi in postmortem cases of septic abortions and stillbirths, highlight the need for an effective preventive strategy, such as vaccination, before or in early pregnancy. However, a better understanding of the responsible NTHi strains through molecular characterization, for example, is needed. Identification of a distinct group associated with perinatal infections could lead to improved diagnostics and possible vaccine target antigens in the future.

Notes

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Author contributions. S. C., M. E. R., M. P. E. S., D. J. L., S. F., and S. N. L. are all involved with enhanced surveillance of invasive \textit{H. influenzae} disease in England and Wales. S. C. was responsible for data collection and maintenance of the surveillance database, and conducted the literature search. S. C. and S. N. L. assessed the literature, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis, wrote the first draft, and coordinated the production of the manuscript. S. N. L. is the guarantor. All authors were involved in the interpretation of the data and writing of the report; all authors approved the final version.

Potential conflicts of interest. S. C., M. E. R., and S. N. L. have provided vaccine manufacturers with postmarketing surveillance reports, which the companies are required to submit to the UK licensing authority in compliance with their risk management strategy; in accordance with Public Health England policy, a cost recovery charge is made for these reports payable to the Immunisation Department. The Respiratory and Vaccine Preventable

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Bacterial Reference Unit received research funding from GlaxoSmithKline (GSK) for clinical and bacteriological study of invasive Hib disease in children (completed 2012). S. N. L. has worked on clinical trials on behalf of St George’s University of London for vaccine manufacturers including GSK and Pfizer, but has received no personal remuneration. M. P. E. S. has received support from Pfizer, GSK, and MSD to attend scientific conferences and has participated in advisory boards and spoken at Pfizer and GSK symposiums during international meetings. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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