Perinatal risk factors for neonatal encephalopathy: an unmatched case-control study

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ABSTRACT
Objective Neonatal encephalopathy (NE) is the third leading cause of child mortality. Preclinical studies suggest infection and inflammation can sensitise or precondition the newborn brain to injury. This study examined perinatal risk factors for NE in Uganda.

Setting Mulago National Referral Hospital, Kampala, Uganda.

Methods 210 term infants with NE and 409 unaffected term infants as controls were recruited over 13 months. Data were collected on preconception, antepartum and intrapartum exposures. Blood culture, species-specific bacterial real-time PCR, C reactive protein and placental histology for chorioamnionitis and funisitis identified maternal and early newborn infection and inflammation. Multivariable logistic regression examined associations with NE.

Results Neonatal bacteraemia (adjusted OR (aOR) 8.67 (95% CI 1.51 to 49.74), n=315) and histological funisitis (aOR 11.80 (95% CI 2.19 to 63.45), n=162) but not chorioamnionitis (aOR 3.20 (95% CI 0.66 to 15.52), n=162) were independent risk factors for NE. Among encephalopathic infants, neonatal case fatality was not significantly higher when exposed to early neonatal bacteraemia (OR 1.65 (95% CI 0.62 to 4.39), n=208). Intrapartum antibiotic use did not improve neonatal survival (p=0.826). After regression analysis, other identified perinatal risk factors (n=619) included hypertension in pregnancy (aOR 3.77), male infant (aOR 2.51), non-cephalic presentation (aOR 5.74), lack of fetal monitoring (aOR 2.75), augmentation (aOR 2.23), obstructed labour (aOR 3.8) and an acute intrapartum event (aOR 8.74).

Conclusions Perinatal infection and inflammation are independent risk factors for NE in this low-resource setting, supporting a role in the aetiological pathway of term brain injury. Intrapartum antibiotic use did not mitigate against adverse outcomes. The importance of intrapartum risk factors in this sub-Saharan African setting is highlighted.

INTRODUCTION
Birth complications and perinatal infections are leading contributors to neonatal mortality globally.1 Each year, peripartum complications contribute to more than one million cases of neonatal encephalopathy (NE) and around half a million survivors with neurological impairment.2 In sub-Saharan Africa, where access to skilled birth attendants and emergency obstetric intervention is often limited, the contribution of peripartum hypoxic events to NE is likely very high.3 Across other settings, however, a number of peripartum risk factors for NE have been identified,4–7 supporting a complex multifactorial model of brain injury.

Increasing evidence suggests the critical importance of a sensitising effect of inflammation in the pathogenesis of NE.8–10 In neonatal rodent studies, exposure to bacterial endotoxin has been found to increase vulnerability of the developing brain to injury, with a pathway involving stimulation of toll-like receptors, inflammatory responses, chemotaxis and cell death.10 Other preclinical studies have shown a temporal relationship between bacterial endotoxin and brain injury, with both sensitising and preconditioning effects seen.11,12 In clinical studies, factors associated with perinatal infection such as maternal fever and prolonged rupture of membranes, are associated with NE.13,14 However, this may be mediated by

What is already known on this topic?

► Perinatal brain injury is the third leading cause of child mortality globally, with some of the highest burden seen in low-resource African settings.
► Preclinical studies suggest infection and inflammation can sensitise or precondition the term newborn brain to injury.
► Understanding which perinatal risk factors are associated with neonatal encephalopathy is key to developing interventions to prevent newborn deaths and disability.

What this study adds?

► Perinatal infection and inflammation are independent risk factors for neonatal encephalopathy in this African population, supporting a role in the aetiological pathway of term brain injury.
► Intrapartum antibiotic use, however, was not associated with improved neonatal outcome.
► The importance of other intrapartum risk factors in this setting is highlighted.
the direct effect of hyperthermia itself on the developing brain as opposed to any underlying cause. Few clinical studies have examined the role of specific perinatal infections and inflammation as independent risk factors for NE, although an important role is hypothesised.

Understanding which perinatal risk factors are associated with NE is key in developing interventions to prevent newborn deaths and disability. Despite a high burden of NE in sub-Saharan Africa, the role of infectious comorbidity, such as neonatal bacteremia and chorioamnionitis, and the contribution of other risk factors in the aetiology of NE have been poorly defined. We conducted an unmatched case–control study among hospital-born newborns in Uganda aiming to identify risk factors for NE in resource-limited settings.

**METHODS AND MATERIALS**

**Setting**

Uganda is a low-income country with a neonatal mortality rate of 23 per 1000 live births. Mulago National Referral Hospital, in the capital Kampala, receives high-risk pregnancies from the city and surrounding areas. In 2012, more than 33,000 deliveries occurred on the low-risk (21%) and high-risk (79%) labour wards. Fetal monitoring is by intermittent auscultation and women are not routinely examined at the start of second stage. Assisted deliveries (ventouse or forceps) are rarely performed. A fifth of deliveries are by caesarean section. Intravenous fluids, antibiotics and oxytocin are available. Neonatal resuscitation, performed by midwives, includes oxygen and bag-mask ventilation. Care on the 80-bed special care baby unit (SCBU) includes simple continuous positive airway pressure ventilation (not mechanical ventilation), intravenous fluids, antibiotics and antiseizure medication. Blood gas estimation facilities are not available.

**Study design and recruitment**

We conducted an unmatched case–control study between September 2011 and October 2012. Written informed parental consent was obtained. All term newborns admitted to the SCBU were examined for encephalopathy using the Thompson score. Cases were term newborns ≥37 weeks, with NE defined as a “Thompson score” >5 within 12 hours of birth, as assessed by CJT or other study doctors. Neurological assessment was performed on recruitment for cases and controls and then daily for 5 days (cases only). Encephalopathy was graded (mild, moderate or severe) on the most severe day between days 1 and 5, per modified Sarnat classification, a scoring system used to grade the severity of hypoxic-ischaemic brain injury. Gestational age was assessed using last menstrual period or early obstetric ultrasound scan, and if unavailable based on external newborn examination. Infants were reviewed after discharge at 4–6 weeks of age to establish survival. Figure 1 describes how case and control infants moved through the study.

To reflect all hospital deliveries, controls were recruited in a ratio of 79:21 from the high-risk and low-risk wards, respectively. Control mothers and infants were systematically sampled from the labour ward admission book. Control infants were eligible for recruitment if term with Thompson score <3. Exclusion criteria (cases and controls) included prior antibiotics given to the infant (which would invalidate blood culture results), mother living >20 km from the hospital, out-born infants and no informed written consent. Infants with congenital abnormalities or other concomitant pathology were not excluded.

**Data collection**

Information from antepartum, intrapartum and postpartum periods was collected using structured maternal interviews and from clinical records. Postnatal anthropometric measurements on mother (height, weight) and newborns (occipitofrontal circumference, birth weight (SECA 336 electronic scales, Hamburg, Germany)) were taken.

**Maternal and neonatal infection and inflammation**

Maternal and neonatal blood was sampled at recruitment (<12 hours of delivery). Blood was stored at −80°C. Maternal and neonatal C reactive protein (CRP) was batch-tested (COBAS, Roche Diagnostics, Basel, Switzerland). Maternal HIV results were recorded from routine hospital testing.

Blood culture and species-specific bacterial real-time PCR assays detected neonatal bacteraemia. Blood cultures (BACTEC) were performed for all case infants and for control infants with a clinical suspicion of sepsis. Isolated colonies were manually identified. Techniques for species-specific bacterial PCR and blood culture among the study cohorts have been published previously. Multiplexed PCR assays for the detection of bacteria considered pathogenic among newborns (group B Streptococcus, Pneumococcus, Staphylococcus aureus, group A streptococcus, Enterobacteriaceae sp) were performed on all cases and the first 101 control infants as a comparison group. CRP was measured as a marker of inflammation and presented according to centiles among control infants.

**Placental pathology**

Placentas are not routinely collected and stored at Mulago Hospital, and we aimed to collect, process and report placental

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**Figure 1** Diagram showing how infants moved through the study procedures. CRP, C reactive protein; qPCR, quantitative Polymerase Chain Reaction.
histology in a quarter of cases and controls. Placentas were collected when infant resuscitation was required (Apgar score <6 at 5 min) and for identified potential controls. Whole placentas were fixed (10% formalin), sampled according to standard protocols and processed (SurgPath, Kampala). Slides were reported by an experienced perinatal pathologist (NJS) at the Camelia Botnar Laboratories, Great Ormond Street Hospital, blinded to outcome and all clinical information. Histological chorioamnionitis and funisitis were defined according to standard criteria.21

Statistical analysis
All data were coded (CJT), double-entered (MS Access) and analysed using Stata V.11.0. Univariable logistic regression was used to calculate crude ORs and 95% CIs to identify the independent effects of variables. A three-phase, causal approach to multivariable modelling was used to estimate adjusted ORs (aORs) using the conceptual framework described in figure 2.

Since the proportion of missing data for variables was small (<5% for all variables, <1% for most variables), extra categories were created to represent missing values for the variables included. Inflammatory factors were defined as maternal CRP >90th centile, histological chorioamnionitis, histological funisitis, positive neonatal bacteremia and raised neonatal CRP >97th centile. Population attributable fraction was calculated to assess the contribution of inflammatory factors to NE. The sample size of 210 cases and 409 controls ensured at least 80% power to detect risk factors conferring OR ≥2.5 at a significance level p<0.05, for main exposures with a prevalence of 5%–80%.

RESULTS
During our 13-month recruitment period, 36926 infants were born at Mulago Hospital, from which 210 encephalopathic and 409 control infants were recruited. Information on the number of babies who were considered as potential cases was not available due to the high number of babies admitted to the SCBU. For controls, a total of 505 mothers of term infants were identified by systemic sampling and approached. Of these 81% (409) consented to recruitment (figure 3).

Table 1 shows the early clinical characteristics of case and control infants. External signs of a major congenital abnormality were uncommon but more frequent in cases (2.9% (6/210) vs 0.7% (3/408), respectively, p=0.037). Losses to follow-up at 4 weeks were 1.0% (2/210) of cases vs 3.2% (13/409) of controls (p=0.103). Neonatal case fatality (<28 days) was 33.7% (n=70, 95% CI 27.2% to 40.1%) among encephalopathic infants (table 1).

Preconception, antepartum and intrapartum risk factors
In univariable analyses (table 2) case mothers were more likely to be primiparous, young, underweight and of short stature, to have severe anaemia or hypertension during pregnancy, and to be HIV-negative. Cases were more likely to be male, have a head circumference >97th centile and non-cephalic presentation. A third of encephalopathic infants were obstructed in labour compared with 8% of controls. Augmentation of labour, prolonged rupture of membranes, prolonged labour and meconium-stained liquor were all significantly more prevalent among cases. Acute intrapartum events were uncommon, but more prevalent among cases. No case infant was delivered by elective caesarean section. Fetal heart rate monitoring was significantly more prevalent among controls. In multivariable analysis one antepartum factor (hypertension in pregnancy), two infant factors (male sex, non-cephalic presentation) and four intrapartum factors (augmentation of labour, no fetal monitoring, acute intrapartum event and obstructed labour) were identified as independent risk factors for NE (table 2).
Perinatal infection/inflammation risk factors

The presence of neonatal bacteraemia was examined in 210 cases and 105 controls. For controls, quantitative polymerase chain reaction (qPCR) was performed among the first 101 recruits. In a further 4, qPCR and blood cultures were performed due to clinical concerns of early possible severe bacterial infection; all were negative. No significant differences were seen between control infants with and without bacteraemia results with respect to demographic or other baseline characteristics (data not shown). The prevalence of pathogenic bacterial species among infants with NE was 3.6%, 6.9% and 8.9%, with culture, PCR and both tests in combination, respectively.20 More encephalopathic infants than controls had pathogenic bacterial species detected (8.9% vs 2.0%, \(p=0.028\)) using culture and PCR in combination.20 PCR detected bacteraemia in 11 culture-negative encephalopathic infants (3 group B Streptococcus, 1 group A Streptococcus, 1 S. aureus and 6 Enterobacteriaceae (2 Enterobacter sp, 1 Pantoea sp, 1 Escherichia coli and 2 identified only as Enterobacteriaceae species)).20 Three case infants were negative on PCR but blood culture was positive for S. aureus. Coagulase-negative staphylococcus was considered not pathogenic.

Table 1 Early clinical characteristics of case and control infants

| Clinical characteristics | Cases n=210 | Controls n=409 | \(p\) Value |
|--------------------------|------------|---------------|-------------|
| Apgar 1 min              |            |               |             |
| ≤3                       | 68/202 (33.7) | 2/405 (0.5)  | <0.0001†  |
| 4–6                      | 116/202 (57.4) | 15/405 (3.7) |             |
| ≥7                       | 18/202 (8.9)  | 388/405 (95.8)|             |
| Apgar at 5 min           |            |               |             |
| ≤3                       | 8/185 (4.3)  | 1/399 (0.25)  | <0.0001†  |
| 4–6                      | 126/185 (68.1) | 1/399 (0.25) |             |
| ≥7                       | 51/185 (27.6) | 397/399 (99.5)|             |
| Need for any resuscitation |                  |               |             |
| No                       | 6/162 (3.7)   | 331/378 (85.1) | <0.001     |
| Yes                      | 156/162 (96.3)| 57/378 (15.1) |             |
| Clinical features         |            |               |             |
| Grade of encephalopathy* |            |               |             |
| Mild                     | 25/210 (11.9) | –             |             |
| Moderate                 | 114/210 (54.3)| –             |             |
| Severe                   | 71/210 (33.8)| –             |             |
| Absent suck              | 166/209 (79.4)| –             |             |
| Clinical seizures        | 104/210 (49.5)| –             |             |
| Comatose                 | 53/210 (25.2)| –             |             |
| Neonatal case fatality   | 70/208 (33.7)| 1/396 (0.3)  | <0.001     |

*Encephalopathy graded according to Sarnat & Sarnat.

Figure 3 Flow diagram of participants.

DISCUSSION

Few studies have examined perinatal risk factors for NE in sub-Saharan Africa. In this Ugandan population, we found that maternal and newborn infection and inflammation, based on blood cultures, molecular assays and a subset of placentas, are independent risk factors for NE, with the strongest associations seen with fetal inflammation (funisitis) and early neonatal bacteraemia. Neonatal case fatality was not significantly higher for infants with early bacteraemia versus those without (44.4% (8/18) with bacteraemia vs 32.6% (62/190) in those without, OR 1.65 (0.62–4.39), \(p=0.32\)). Intrapartum antibiotic was commonly used in cases and controls (17.8% (37/210) vs 14.0% (57/408), respectively, \(p=0.213\)). Among encephalopathic infants, intrapartum antibiotic use was not associated with improved neonatal survival (case fatality with intrapartum antibiotics 32.4% (12/37) vs 34.3% (58/169) without, \(p=0.826\)).
In our study, funisitis, or infiltration of the umbilical cord with acute fetal inflammatory cells, was a significant risk factor for NE; however, the presence of membrane inflammation alone (isolated histological chorioamnionitis) was not. The reduced sample size of infants with placentas collected (n=162) may have been responsible for the lack of significance of the association seen between NE and chorioamnionitis; however, this smaller sample size retained more than 80% power to detect the associations seen for funisitis. Triggering of the fetal inflammatory response is a key event, or that duration of exposure, or proximity of inflammation to the fetus, may be important.

Table 2  Risk factors associated with neonatal encephalopathy in univariable analysis and after adjustment for preconception, antepartum and intrapartum factors

| Risk factor                      | Cases n (%) | Controls n (%) | Unadjusted OR (n=620) | 95% CI | Adjusted OR†† (n=614) | 95% CI |
|---------------------------------|-------------|----------------|-----------------------|-------|-----------------------|-------|
| **Sociodemographic factors**    |             |                |                       |       |                       |       |
| Socioeconomic group             |             |                |                       |       |                       |       |
| High                            | 33/209 (15.8)| 82/405 (20.3)  | 1.00                  | 1.00  |                       | 1.00  |
| Medium                          | 131/209 (62.7)| 245/405 (60.5) | 1.64                  | 0.87 to 3.11 | 1.52           | 0.81 to 2.34 |
| Low                             | 45/209 (21.5)| 78/405 (19.3)  | 1.46                  | 0.83 to 2.47 | 1.39           | 0.64 to 3.01 |
| **Preconception factors**       |             |                |                       |       |                       |       |
| Maternal age <20 years          | 61/210 (29.1)| 76/408 (18.6)  | 1.79                  | 1.21 to 2.64 | 1.28           | 0.70 to 2.50 |
| Maternal weight <50 kg          | 31/208 (14.9)| 34/401 (8.5)   | 1.89                  | 1.13 to 3.18 | 0.69           | 0.32 to 1.61 |
| Maternal height <150 cm         | 40/208 (19.2)| 44/399 (11.0)  | 1.92                  | 1.21 to 3.06 | 1.48           | 0.73 to 3.00 |
| **Antepartum factors**          |             |                |                       |       |                       |       |
| Primiparity                     | 123/210 (58.6)| 183/409 (44.7) | 1.75                  | 1.25 to 2.44 | 1.56           | 0.86 to 2.84 |
| ≥4 Antenatal visits             | 99/210 (47.1)| 225/408 (55.2) | 0.73                  | 0.52 to 1.01 | 0.96           | 0.60 to 1.52 |
| Previous ‘birth asphyxia’       | 208/7 (23.0)| 33/226 (14.6)  | 1.75                  | 0.94 to 3.25 | 1.27           | 0.50 to 3.21 |
| Previous perinatal death        | 24/87 (27.6)| 44/226 (19.5)  | 1.58                  | 0.89 to 2.80 | 1.22           | 0.52 to 2.91 |
| Severe anaemia during pregnancy*| 10/209 (4.8)| 5/408 (1.2)    | 4.05                  | 1.37 to 12.01 | 4.38           | 0.97 to 19.82 |
| Hypertension during pregnancy†  | 18/210 (8.6)| 17/408 (4.2)   | 2.16                  | 1.09 to 4.28 | 3.77           | 1.49 to 9.55 |
| Maternal HIV-positive           | 15/210 (7.1)| 53/409 (13.0)  | 0.52                  | 0.28 to 0.94 | 0.57           | 0.24 to 1.32 |
| **Infant factors**              |             |                |                       |       |                       |       |
| Male                            | 136/210 (64.8)| 196/409 (47.9) | 2.00                  | 1.42 to 2.82 | 2.51           | 1.55 to 4.07 |
| Birth weight <2.5 kg            | 11/209 (5.3)| 27/409 (6.6)   | 0.81                  | 0.39 to 1.67 | 0.46           | 0.15 to 1.44 |
| Birth weight >4.0 kg            | 12/209 (5.7)| 12/409 (2.9)   | 2.00                  | 0.88 to 4.51 | 2.17           | 0.77 to 6.13 |
| Large head circumference‡       | 21/191 (11.0)| 120/400 (3.0)  | 3.99                  | 1.92 to 8.30 | 2.28           | 0.59 to 6.22 |
| Twins                           | 5/210 (2.4)| 13/409 (3.2)   | 0.74                  | 0.26 to 2.11 | 0.58           | 0.13 to 2.61 |
| Non-cephalic presentation       | 23/210 (10.9)| 11/409 (2.7)   | 4.47                  | 2.14 to 9.37 | 5.74           | 2.01 to 16.41 |
| **Intrapartum factors**         |             |                |                       |       |                       |       |
| Augmentation of labour          | 42/209 (20.1)| 43/408 (10.5)  | 2.13                  | 1.34 to 3.39 | 2.23           | 1.17 to 4.23 |
| No auscultation of fetal heart rate during labour | 114/208 (54.8) | 115/408 (28.2) | 3.09                  | 2.18 to 4.38 | 2.75           | 1.71 to 4.22 |
| Prolonged rupture of membranes† | 21/193 (10.9)| 15/396 (3.8)   | 3.10                  | 1.56 to 6.16 | 2.44           | 0.90 to 6.60 |
| Acute intrapartum event‡        | 8/210 (3.8)| 4/409 (1.0)    | 4.01                  | 1.19 to 13.47 | 8.74           | 1.70 to 45.02 |
| Obstructed labour†              | 65/181 (35.9)| 33/407 (8.1)   | 6.35                  | 4.00 to 10.14 | 3.80           | 1.96 to 7.36 |
| Prolonged labour††              | 100/190 (52.6)| 157/387 (40.6) | 1.63                  | 1.15 to 2.31 | 0.81           | 0.47 to 1.39 |
| Meconium-stained liquor         | 55/121 (45.5)| 19/359 (5.3)   | 14.91                 | 8.31 to 26.75 | #              | – |
| Elective caesarean section      | 0/209 (0.0)| 3/409 (0.7)    | –                     | –     | #                     | –     |
| Emergency caesarean section     | 50/209 (23.9)| 56/409 (13.7)  | 1.98                  | 1.30 to 3.03 | #              | –     |

*Defined as haemoglobin ≤7 g/dL during pregnancy and documented in the clinical record.
†Defined as systolic blood pressure >140 mm Hg or diastolic >90 mm Hg developing after 20 weeks’ gestation.
‡Defined as >97th centile in the control population (37.9 cm).
§Defined as rupture ≥24 hours.
‖Defined as antepartum haemorrhage, cord prolapse or uterine rupture.
**Defined as labour with no advance of the presenting part despite strong, regular uterine contractions as documented in the intrapartum record.
¶Defined as antepartum haemorrhage, cord prolapse or uterine rupture.
‖‖Adjusted for all other variables in the table plus maternal C reactive protein >90th centile and neonatal bacteraemia. Factors considered a consequence of intrapartum events (#) were not included in the model.

In our study, funisitis, or infiltration of the umbilical cord with acute fetal inflammatory cells, was a significant risk factor for NE; however, the presence of membrane inflammation alone (isolated histological chorioamnionitis) was not. The reduced sample size of infants with placentas collected (n=162) may have been responsible for the lack of significance of the association seen between NE and chorioamnionitis; however, this smaller sample size retained more than 80% power to detect the associations seen for funisitis. Triggering of the fetal inflammatory response is a key event, or that duration of exposure, or proximity of inflammation to the fetus, may be important.

Reported (22%–31%), with one retrospective observational study also finding funisitis to be significantly associated with encephalopathy (OR 9.29). A recent study from the Netherlands, examining associations between placental pathology and pattern of brain injury in NE, found a high incidence of histological chorioamnionitis across all patterns of brain injury, when compared with healthy term deliveries in a historic cohort from the same centre (50% vs 18%, respectively). The stronger associations with NE seen for histological funisitis, as opposed to chorioamnionitis, may imply that activation of the fetal inflammatory response is a key event, or that duration of exposure, or proximity of inflammation to the fetus, may be important.
Table 3 Univariable and adjusted analyses of perinatal infection/inflammation risk factors and between inflammatory and probable asphyxial factors

| Risk factor                              | Case n (%) | Control n (%) | Univariable analysis | Multivariable analysis |
|------------------------------------------|------------|---------------|----------------------|------------------------|
| Maternal and newborn C reactive protein (CRP) |            |               |                      |                        |
| Maternal CRP                             |            |               |                      |                        |
| <10th centile (≤4.7 mg/L)                | 0/392 (0.5) | 0/392 (0.5)   | 1.00 –               | 1.00 –                 |
| 10th–90th centile (4.7–86.6 mg/L)        | 34/392 (8.7)| 34/392 (8.7)  | 1.10 to 21.22        | 2.57 to 30.92          |
| >90th centile (>86.6 mg/L)               | 20/392 (5.1)| 20/392 (5.1)  | 1.00 –               | 1.00 –                 |
| Neonatal CRP                             |            |               |                      |                        |
| <90th centile (<6.6 mg/L)                | 0/392 (0.5) | 0/392 (0.5)   | 1.00 –               | 1.00 –                 |
| 90th–97th centile (6.6–31.7 mg/L)        | 0/392 (0.5) | 0/392 (0.5)   | 1.00 –               | 1.00 –                 |
| >97th centile (>31.7 mg/L)               | 0/392 (0.5) | 0/392 (0.5)   | 1.00 –               | 1.00 –                 |
| Maternal and newborn infection and inflammation |          |               |                      |                        |
| Neonatal bacteraemia*                    | 18/210 (8.6)| 2/105 (1.9)   | 4.83                 | 1.10 to 21.22          |
| Histological chorioamnionitis alone (no funisitis) | 10/60 (16.7)| 1/102 (1.0)   | 2.33                 | 0.91 to 5.98           |
| Histological funisitis (with chorioamnionitis) | 16/60 (26.7)| 4/102 (3.9)   | 10.24                | 3.19 to 32.82          |

*Defined as blood culture and/or species-specific bacterial quantitative Polymerase Chain Reaction (qPCR) for an organism known to be pathogenic among term newborns.
†Adjusted for preconception/antepartum factors in table 2, plus intrapartum factors (augmentation of labour, prolonged labour, obstructed labour and acute intrapartum event).

Both a sensitising and preconditioning role of bacterial endotoxin on the effect of hypoxia-ischaemia on the immature brain have been seen in animal models, but supporting clinical data are limited. Our study provides evidence of an important role for early newborn bacteraemia in NE in humans, with an eightfold increase in odds of NE. Early neonatal infection did not significantly increase the odds of death among infants with NE, although the small numbers of infants in the infection-exposed group (n=18) may have reduced the ability to detect a true difference. Our findings are supported, however, by a US study, examining MRI in predicting outcome after NE, that reported a similar prevalence of sepsis and an increase in adverse neurodevelopmental outcomes, but not death, among encephalopathic infants with sepsis.27 Despite the administration of intrapartum antibiotics in almost a fifth of encephalopathy cases in our study, antibiotics did not improve survival. This lack of effect of antibiotic therapy is consistent with findings from obstetric intervention may have been substantial contributing factors in our setting. Prioritising effective risk assessment and intrapartum care for women and babies delivering in healthcare facilities in low-resource settings has the potential to substantially reduce neonatal risk.35

There were limitations to this study. Placental sampling was performed in only a quarter of women, and although sampling appeared non-differential all bias cannot be excluded, and power to detect associations for placental variables was reduced. Recruitment of participants from a high-risk referral centre may explain the high rates of chorioamnionitis seen; however, this would have led to a conservative estimate of all ORs. Although we performed species-specific bacterial PCR in conjunction with neonatal blood culture to strengthen the diagnosis of neonatal bacteraemia, we still cannot exclude underdiagnosis. The majority of control infants did not show clinical signs of sepsis, and loss to follow-up rates in both groups were low. We cannot exclude all selection and recall bias, although rigorous...
verification with hospital records and comprehensive staff interview training aimed to minimise this.

In summary, perinatal infection and inflammation are independent risk factors for NE in this low-resource setting, supporting a role in the aetiological pathway of term brain injury. Intrapartum antibiotic administration did not mitigate again adverse outcomes. The importance of intrapartum risk factors in this sub-Saharan African setting is highlighted.

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Contributors CJT, JJK, NK, NJS, DP, KA H, AME and NJR designed the study and were involved in data interpretation and report writing. CJT implemented and led the study. Data analysis was conducted by CJT and BAW, ELW and JJK provided statistical support. CJT, MN, MS, MM, NN, IO, EDM and PN participated in data collection. CJT, PN and KA H performed the molecular assays. MN, JM, AME and NJR provided logistical support. All authors reviewed and approved the final version. All authors had full access to all of the data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. CJT affirms that the manuscript is an honest, accurate and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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REFERENCES
1. Liu L, Oza S, Hogan D, et al. Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet 2015;385:40–40.
2. Lee AC, Kozuki N, Blencowe H, et al. Intrapartum-related neonatal encephalopathy incidence and impairment at regional and global levels for 2010 with trends from 1990. Pediatr Res 2013;74(Suppl 1):50–72.
3. Laws KE, Lee AC, Kinney M, et al. Two million intrapartum-related stillbirths and neonatal deaths: where, why, and what can be done? Int J Gynaecol Obstet 2009;107(Suppl 1):55–59.
4. Badawi N, Kuninzzik JJ, Kehg JM, et al. Antepartum risk factors for newborn encephalopathy: the Western Australian case-control study. BMJ 1998;317:549–53.
5. Badawi N, Kuninzzik JJ, Kehg JM, et al. Intrapartum risk factors for newborn encephalopathy: the Western Australian case-control study. BMJ 1998;317:549–53.
6. Ellis M, de L Costello AM. Antepartum risk factors for newborn encephalopathy. Intrapartum risk factors are important in developing world. BMJ 1999;318:1414.2
7. Martinez-Biarge M, Diez-Isacatian J, Wushoff CJ, et al. Antepartum and intrapartum factors preceding neonatal hypoxic-ischemic encephalopathy. Pediatrics 2013;132:e952–e959.
8. Fleiss B, Tann CJ, Depug V, et al. Inflammation-induced sensitization of the brain in term infants. Dev Med Child Neurol 2015;57(Suppl 3):17–28.
9. Hagberg H, Mailard C, Ferriero DM, et al. The role of inflammation in perinatal brain injury. Nat Rev Neurol 2015;11:192–208.
10. Stridh L, Mottahedin A, Johansson ME, et al. Toll-like receptor-3 activation increases the vulnerability of the neonatal brain to hypoxia-ischemia. J Neurosci 2011;31:8456–63.
11. Eklind S, Mailard C, Avdkinson P, et al. Lipopolysaccharide induces both a primary and a secondary phase of sensitization in the developing rat brain. Pediatr Res 2005;58:112–6.
12. Stevens SL, Leung PY, Vartanian KB, et al. Multiple preconditioning paradigms converge on interferon regulatory factor-dependent signaling to promote tolerance to ischemic brain injury. J Neurosci 2011;31:8456–63.
13. Peebles D. Intrauterine infection and perinatal brain injury. Royal College of Obstetrics & Gynaecology: Scientific Advisory Committee opinion paper, 2007.
14. Nelson KB, Penn AA. Is infection a factor in neonatal encephalopathy? Arch Dis Child Fetal Neonatal Ed 2015;100:F8–F10.
15. UNICEF. Levels and trends in child mortality, report. New York, USA: UNICEF; WHO, The World Bank, United Nations, 2013.
16. Thompson CM, Pottermaker AS, Linkey LJ, et al. The value of a scoring system for hypoxic ischemic encephalopathy in predicting neurodevelopmental outcome. Acta Paediatr 1997;86:757–61.
17. Samat H, Samat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Arch Neurol 1976;33:696–705.
18. Parkin JM, Hey EN, Clowe JS. Rapid assessment of gestational age at birth. Arch Dis Child 1976;51:259–63.
19. Namikawa Z, Fukunishi T, Kato T, Peikinlinna A, et al. The impact of maternal highly active antiretroviral therapy and short-course combination antiretrovirals for prevention of mother-to-child transmission on early infant infection rates at the Mulago national referral hospital in Kampala, Uganda, January 2007 to May 2009. J Acquir Immune Defic Syndr 2011;56:69–75.
20. Tann CJ, Nkurunziza P, Nakaeto M, et al. Prevalence of bloodstream pathogens is higher in neonatal encephalopathy cases vs. controls using a novel panel of real-time PCR assays. PLoS One 2014;9:e97259.
21. H van F. Pathology of placentas. Philadelphia, USA: Elsevier Limited, 2007.
22. Redline RW. Inflammatory response in acute choiromionitis. Semin Fetal Neonatal Med 2012;17:20–5.
23. Redline RW. Inflammatory responses in the placenta and umbilical cord. Semin Fetal Neonatal Med 2006;11:296–301.
24. Hayes BC, Cooley S, Donnelly J, et al. The placenta in infants >36 weeks gestation with neonatal encephalopathy: a case control study. Arch Dis Child Fetal Neonatal Ed 2013;98:F233–F239.
25. Lau J, Magee F, Qiu Z, et al. Choioriomnitis with a fetal inflammatory response is associated with higher neonatal mortality, morbidity, and resource use than choioriomnitis displaying a maternal inflammatory response only. Am J Obstet Gynecol 2005;193:708–13.
26. Herteman IC, Nikkels PG, Benders MJ, et al. Placental pathology in full-term infants with hypoxic-ischemic neonatal encephalopathy and association with magnetic resonance imaging pattern of brain injury. J Pediatr 2013;163:968–75.
27. Jenster M, Bonfascio SL, Ruel T, et al. Maternal or neonatal infection: association with neonatal encephalopathy outcomes. Pediatr Res 2014;76:93–9.
28. Kenyon SL, Taylor DJ, Tarnow-Mordi W. Broad-spectrum antibiotics for preterm, prelabour rupture of fetal membranes: the ORACLE I randomised trial. ORACLE Collaborative Group. Lancet 2001;357:979–88.
29. Kenyon SL, Taylor DJ, Tarnow-Mordi W. Broad-spectrum antibiotics for spontaneous preterm labour: the ORACLE II randomised trial. ORACLE Collaborative Group. Lancet 2001;357:989–94.
30. Jacobs SE, Berg M, Hunt R, et al. Cooling for newborns with hypoxic ischaemic encephalopathy. Cochrane Database Syst Rev 2013:Cd003311.
31. Iwamoto A, Seward N, Prost A, et al. Maternal infection and risk of intrapartum death: a population based observational study in South Asia. BMC Pregnancy Childbirth 2013;13:245.
32. Nelson KB, Bingham P, Edwards EM, et al. Antecedents of neonatal encephalopathy in the Vermont Oxford Network Encephalopathy Registry. Pediatrics 2012;130:878–86.
33. Nelson KB, Grether JK. Potentially asphyxiating conditions and spas tic cerebral palsy in infants of normal birth weight. Am J Obstet Gynecol 1998;179:507–13.
34. Hofer N, Zacharias E, Müller W, et al. An update on the use of C-reactive protein in early-onset neonatal sepsis: current insights and new tasks. Neonatology 2012;102:25–36.
35. Bhutta ZA, Das JK, Bahl R, et al. Can available interventions end preventable deaths in mothers, newborn babies, and stillbirths, and at what cost? Lancet 2014;384:347–70.