Article type: Original article**Incidence and Risk Factors for Neonatal Jaundice among Newborns in Southern Nepal**

Carolyn G. Scraftford¹, Luke C. Mullany¹, Joanne Katz¹, Subarna K. Khatri^{1,2}, Steven C. LeClerq^{1,2}, Gary L. Darmstadt³, James M. Tielsch⁴

1 Department of International Health, Global Disease Epidemiology and Control, Johns Hopkins Bloomberg School of Public Health, Baltimore MD, USA

2 Nepal Nutrition Intervention Project – Sarlahi, Kathmandu, Nepal

3 Family Health Program, Global Development Division, Bill and Melinda Gates Foundation, Seattle, WA, USA

4 Department of Global Health, School of Public Health and Health Services, George Washington University, Washington DC, USA

ABSTRACT

Objective: To quantify the incidence of and risk factors for neonatal jaundice among infants referred for care from a rural, low-resource, population-based cohort in southern Nepal.

Methods: Study participants were 18,985 newborn infants born in Sarlahi District in Southern Nepal from May 2003 through January 2006 who participated in a cluster-randomized, placebo-controlled, community-based trial to evaluate the effect of newborn chlorhexidine cleansing on neonatal mortality and morbidity. Jaundice was assessed based on visual assessment of the infant by a study worker and referral for care. Adjusted relative risks (RR) were estimated to identify risk factors for referral for neonatal jaundice using Poisson regression.

Results: The incidence of referral for neonatal jaundice was 29.3 per 1,000 live births (95% Confidence Interval: 26.9, 31.7). Male sex, high birth weight, breastfeeding patterns, warm air temperature, primiparity, skilled birth attendance, place of delivery, prolonged labor, oil massage, paternal education, and ethnicity were significant risk factors (p -values <0.01). After multi-variable adjustment, sex, birth weight, difficulty feeding, prolonged labor, primiparity, oil massage, ambient air temperature, and ethnicity remained important factors. Among infants with difficulty feeding, exclusive breastfeeding was a risk factor for neonatal jaundice, whereas exclusive breastfeeding was protective among infants with no report of difficulty feeding.

Conclusions: Several known risk factors for neonatal jaundice in a low-resource setting were confirmed in this study. Unique observed associations of jaundice with ambient air temperature and oil massage may be explained by the opportunity for phototherapy based on the cultural practices of this study population. Future research should investigate the role of an infant's difficulty in feeding as a potential modifier in the association between exclusive breastfeeding and jaundice.

Keywords: neonatal jaundice, risk factors, Nepal, developing country

INTRODUCTION

Neonatal jaundice is a common physiological occurrence in newborns with over half of term (1,2) and 80% of preterm neonates (3,4) showing clinical signs including yellow discoloration of the skin and sclera resulting from high serum levels of bilirubin. Jaundice is a result of the increased breakdown of red blood cells and/or decreased hepatic excretion of bilirubin. For the majority of these infants, hyperbilirubinemia is a natural transition that resolves within the first week of life with maturing of the liver; however, hyperbilirubinemia is also the main reason for hospital readmission during the neonatal period(5-7). Hyperbilirubinemia is a primary concern associated with jaundice due to the connection between increased levels of unconjugated bilirubin and neurotoxic effects causing long-term sequelae including cerebral palsy, hearing loss, and kernicterus(4, 8, 9).

Of the 7.6 million deaths estimated to occur in children under the age of 5 years in 2010, 40% occurred during the neonatal period(10). Given Millennium Development Goal 4 to reduce under-5 child mortality by two-thirds by 2015(11), attention needs to be focused on the neonatal period and associated morbidity and mortality. Several reports have indicated the important contribution of severe neonatal jaundice and hyperbilirubinemia to neonatal morbidity and mortality(6, 7, 12-15). In a multi-center study in six developing countries, hyperbilirubinemia was a primary diagnosis for severe illness requiring hospital admission, the cause for 12-78% of the admissions in the first 6 days of life and for 2-57% of admissions during the next 7-59 days(15). While the majority of infants have serum levels of 5-6 mg/dl and will not progress to hyperbilirubinemia, higher levels have been found in exclusively breast-fed infants (16-18) and in areas of the world where glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is prevalent. This enzyme deficiency coincides with regions with higher child mortality rates, such as Africa and South Asia(19, 20).

In low-resource settings, where the majority of births occur outside facilities and access to monitoring and laboratory testing is limited, many of the proven diagnostic and treatment strategies are not available. Worldwide, an estimated 14.1 million newborns (10.5% of live births) require phototherapy for jaundice; of these, 6 million do not have access to treatment and 2.4 million without access to treatment are in Central and South Asia(21).

This study aimed to provide information on the incidence and risk factors associated with neonatal jaundice in a low-resource setting with the objective of identifying potentially modifiable risk factors to reduce the incidence and consequences of jaundice in neonates.

METHODS

Data Collection

Data in this analysis were collected as part of a nested pair of cluster-randomized, placebo-controlled, community-based trials conducted to evaluate the impact of whole body skin cleansing and treatment of the umbilical cord with chlorhexidine on neonatal mortality and morbidity. The trials were conducted from September 2002 through January 2006 in southern Nepal by the Nepal Nutrition Intervention Project – Sarlahi (NNIPS) and the study population and procedures have been described previously(22, 23). Pregnant women at around 6 months gestation were enrolled, received weekly vitamin A supplements, albendazole, tetanus immunization, and were given a clean birthing kit and education on proper nutrition, clean delivery, and basic umbilical cord and thermal care during and post-delivery. Newborns were cluster-randomized to receive a full-body cleansing regime using 0.25% chlorhexidine solution (clusters = 206) or placebo (clusters=207)(23) and then subsequently 1 of 3 cord care treatments: dry cord care, soap/water, or 4% chlorhexidine solution(22). The experimental washing study lasted from September 2002 through March 2005, at which time all newborns received the chlorhexidine treatments on recommendation of the trial's Data and Safety Monitoring Board.

Household level data collected during a household interview included socioeconomic status, education, reproductive history, and household structure. Study staff visited each newborn up to 11 times during the neonatal period (days 1,2,3,4,6,8,10,12,14,21,28). During these visits, morbidity signs and vital status were recorded. Data on the delivery process, condition, and immediate care of the newborn were collected on day 1 including birth weight (using a digital infant scale to +/- 2 g), gestational age (time between birth and date of last menstrual period, as reported by the mother at enrollment), length of labor, birth injuries, color at birth, birth location, and presence of a skilled birth assistant. Data on newborn care practices were collected on days 1 and 14 including time to initiation of breastfeeding, oil massage given, and thermal care. Air temperature on the infant's birth date was calculated as the average of the daily minimum ambient air temperature recorded at two local airports adjacent to the study area (Simara and Janakpur.

Case Definition of Incident Neonatal Jaundice

A formal referral and data collection process was initiated on May 15, 2003. Any infant born before May 15, 2003 was excluded from the current analysis. Study workers could refer a newborn for up to three illnesses or conditions reported by the newborn's family or identified by the worker upon examination of the child on any of the 11 follow-up visits. Incident neonatal jaundice was based on visual assessment only of the infant and was defined as the first report of "yellow body/eyes" at any time during follow-up.

Statistical Analysis

Potential risk factors for neonatal jaundice were grouped into three categories: 1) infant; 2) intra-partum and maternal; and 3) household and socioeconomic factors. All potential risk factors were analyzed separately and comparisons of those newborns referred for jaundice to those with no referrals or referrals for conditions other than jaundice were conducted using Poisson regression with the Huber-White sandwich estimator for robust standard error(24) to estimate the relative risk (RR). We further evaluated adjusted associations in multivariable models, adding risk factors in groups. Model 1 included the most proximal infant risk factors: sex, birth weight, gestational age, and small for gestational age (SGA). Model 2 added intermediate factors: place of delivery, skilled birth attendant present, breastfeeding practices, injury at birth, oil massage, air temperature, maternal age, parity, and labor and delivery variables. The final model (Model 3) added distal household and socioeconomic factors.

Maternal report of the infant having difficulty feeding or sucking was assessed over the same period of follow-up as the referral process. In bivariate analyses, an infant reported to have difficulty feeding over any of the 11 morbidity follow-up days was determined to have that risk factor. To adjust for timing of report of difficulty feeding and its temporal association with referral for jaundice, we conducted a nested case-cohort analysis. In this analysis, for every infant referred for jaundice on day x (where x ranged over the possible visit days), 10 controls were randomly selected among infants who were not referred by day x and matched to cases based on the proportion of the 11 potential follow-up days they were visited by day x . The exposure for each case and the controls was defined as maternal report of difficulty feeding for any day prior to selection as a case or control. Conditional logistic regression techniques were used to estimate odds ratios (OR).

All analyses were adjusted for the intervention and the cluster-randomized design of the treatment allocation in the parent trial and conducted using STATA 10.0 (Stat Corp, College Station, TX). The study was approved by the Nepal Health Research Council (Kathmandu, Nepal) and Johns Hopkins Bloomberg School of Public Health Committee on Human Research. The parent trial is registered at <http://www.Clinicaltrials.gov> (NCT00109616).

RESULTS

Of the 23,662 infants born in the study area during this trial, 18,985 (80.2%) were born after May 15, 2003 and included in our analysis. The median number of follow-up days per infant was 10. The overall characteristics of the study population have been described previously(23). The incidence of referral for neonatal jaundice was 29.3 per 1000 live births ($n=556$) (95% CI: 26.9 -31.7). The median age of the infant when referred for “yellow body/eyes” was 6 days (range <1 day to

32.7 days). Approximately half of the incidence occurred within the first week of life (N=310 (55.8%); Figure 1).

In bivariate analyses (Table 1), male infants, infants with high birth weight (≥ 3000 grams compared to 2500-2999g), infants with difficulty feeding, infants who were given colostrum, born in a facility, had a skilled attendant at the delivery, were blue/grey at birth, or had an injury at birth, and infants with mothers who reported vaginal bleeding 7 days prior to delivery or a prolonged labor were significantly more likely to be referred for jaundice. Alternatively, infants who were not exclusively breastfed, began to breastfeed at ≥ 24 hours of age, and received a mustard oil massage were less likely to be referred for jaundice. Season of birth was significantly associated with neonatal jaundice with an increased risk observed among those infants born in the “hot season” (March – October) and was further validated by the recorded minimum ambient air temperature on the infant’s birthdate which indicated a significant 3% increase in risk for each 1°C increase in ambient temperature.

Infants with older mothers were progressively less likely to be referred for each 5-year increase in age than mothers <20 years of age. Mothers who had ≥ 1 previous live born babies were less likely to have an infant referred for jaundice. Jaundice increased significantly with several indicators of higher socioeconomic status including parental literacy and education. Electricity, television, and a latrine in the household were also associated with a higher risk of referral for jaundice. Infants of *madeshi* ethnicity (originating from the plains) had a decreased risk of jaundice compared to infants of *pahadi* (originating from the hills) ethnicity [RR=0.21 (95%CI: 0.18-0.25)].

Multivariable models were constructed with potential risk factors added in groups in the order of proximate, intermediate, and distal factors (Table 2). Model 1 included infant factors that after adjustment showed little change in RR estimates with the exception of an attenuated risk estimate for SGA. Male sex was the only risk factor that remained significantly associated with an increased risk of jaundice.

In Model 2, maternal and intrapartum factors were added. Significant collinearity between exclusive breastfeeding and time to initiation of breastfeeding has been reported in this study population(25). Given the setting of this study and the importance of exclusive breastfeeding to jaundice risk, time to initiation of breastfeeding was not included in the multi-variable models. Additionally, we restricted Model 2 to ambient air temperature given the strength of this variable in analyses including season of birth. Infant risk estimates in Model 2 were slightly attenuated after further adjustment with the exception of birthweight where there was a slight increase in risk in the low and high birthweight groups. Exclusive breastfeeding, difficulty feeding, skilled birth at-

tendant present, color at birth, oil massage, air temperature, primiparity, and prolonged labor remained significantly associated with jaundice in Model 2. A significant interaction between exclusive breastfeeding and difficulty feeding was observed (p-value for interaction = 0.006). Among infants with difficulty feeding, exclusive breastfeeding showed an increased risk for jaundice [RR=1.28 (95%CI: 0.89-1.84)]. However, among infants with no report of difficulty feeding, exclusive breastfeeding was protective [RR=0.72 (95%CI: 0.58-0.88)]. Results presented in Models 2 and 3 are adjusted for this interaction.

Model 3 added household and socioeconomic factors. Parental variables were restricted to schooling years and occupation; parental literacy was not included due to strong with education. Infant sex, birthweight, difficulty feeding, oil massage, ambient air temperature, primiparity, prolonged labor, improved latrine, and *pahadi* ethnicity were significantly associated with jaundice after adjustment for all measured potential risk factors in this study while exclusive breastfeeding, skilled birth attendant, and color at birth were no longer significant factors. Estimates of risk for sex, birthweight, difficulty feeding, oil massage given, minimum ambient air temperature, parity, prolonged labor, and ethnicity remained relatively unchanged throughout the analyses regardless of adjustment for other factors.

The results of the nested case-cohort analysis to adjust for potential reverse causation bias in the association between difficulty feeding and neonatal jaundice are presented in Table 3. The significant association between difficulty feeding and increased risk for neonatal jaundice was still significant and stronger when the analysis was restricted to those infants where difficulty feeding preceded referral for neonatal jaundice.

DISCUSSION

Approximately 3% of newborn infants born in our study population in southern Nepal were referred for neonatal jaundice in the first month of life. Worldwide, it is estimated that 10.5% of live births require phototherapy for jaundice and Nepal estimates are in the range of 3-6%(21). Research from a hospital-based study in Dharan (Eastern), Nepal found that 9.2% of infants admitted to the neonatal intensive care unit (NICU) had pathologic jaundice(26). Estimates from recent studies show 6.7% in Lagos, Nigeria(13) and 10.5% and 25.3% in term and near-term (35-37 weeks) newborns, respectively, in Turkey(2). However, there is no standard definition of jaundice and the endpoint varies significantly between studies. In our study, the definition of neonatal jaundice was based on referral by a study worker observing the infant during morbidity follow-up visits with instruction to refer based on yellowness of the body and/or eyes or maternal report. It is very likely that this definition was not sufficiently sensitive and cases of jaundice were missed

during this period. This under-ascertainment has been observed in one study evaluating the World Health Organization's (WHO's) Integrated Management of Childhood Illness (IMCI), in which jaundice was frequently missed(27). The authors also reported improved diagnostic accuracy when the lower extremities, including the soles of the feet, were included in the assessment. We noted a significant increase in risk with an increase in ambient air temperature which may be explained, in part, by the fewer clothes worn by newborns in the hot season and the worker's increased ability to observe an infant's lower extremities. Sero-concentration of bilirubin due to heat-induced increases in transepidermal water loss, and thus an increased degree of dehydration, which is well-known to be associated with increased expression of jaundice, might also be a factor. In addition, many of the reported incident and prevalence measures of jaundice in the literature based on bilirubin measurements in the blood use various cut-offs for hyperbilirubinemia, creating a lack of standardization and comparability in these estimates.

Serum bilirubin levels were not measured in our study due to the fact that this was a secondary analysis of primary data collected from a larger randomized controlled trial that was not designed with the primary objective of measuring bilirubin levels in neonates and assessing incident jaundice; our estimate of the incidence of referral for neonatal jaundice is limited to a measure of the physical signs of jaundice as recognized by study workers and family members. This is a major limitation of this study since visual evaluation of jaundice can be misleading, especially in infants with darkly pigmented skin (28). Transcutaneous biliurubin (TcB) measurements are suggested to be a potential non-invasive surrogate for serum levels without requiring blood to be taken from an infant or access to a laboratory (16). A recent study in north India among healthy term and late preterm neonates showed that TcB along with gestation at birth and parity status were good predictors of pathological hyperbilirubinemia (29). Transcutaneous bilirubinometers correlate well with serum bilirubin and to be valid indicators of hyperbilirubinemia in preterm and term neonates (30). There are also relatively inexpensive instruments such as the icterometer and the jaundicemeter that are non-invasive diagnostic tools which have been used for many years to improve the visual assessment of jaundice in neonates and could be used in community-based studies in low-resource settings. However, the sustainability and cost of supplies, as well as the training of workers in low-resource and non-facility based settings to appropriately interpret the results would need to be considered.

The main objective of our study was to identify potentially modifiable and recognizable risk factors to allow for screening at the community level to identify those newborns at risk and in turn help to guide appropriate referral and increase the appropriate treatment of neonatal. Further

evaluation of community-based diagnosis of neonatal jaundice should be researched in settings similar to the one in this study.

This study confirmed the importance of several known risk factors for jaundice in neonates including birthweight, difficulty feeding, male sex, and primiparity(5, 13, 16, 31-36). Prolonged labor was strongly associated with jaundice in our study population; most likely due to the clinical relationship between longer labor and cephalohematoma, a known risk factor for severe hyperbilirubinemia(3, 37). Several established factors in the literature were not important risk factors in our study population including gestational age and twins(5, 13, 31, 32, 35, 38, 39). It is well documented that there is an increasing risk for significant hyperbilirubinemia with decreasing gestational age (2, 3, 6, 18, 37, 38, 40) along with a disproportionate number of late pre-term infants accounting for hospital readmissions for management of jaundice and hyperbilirubinemia(41). In our study we relied on family members' ability to recognize and report "yellow body/eyes" in their infant, which most likely varied greatly. Therefore we were unable to replicate prior research associating increasing serum bilirubin levels with decreasing gestational age (e.g., serum levels among infants born at 40 weeks gestational age compared to infants born at 39, 38, 37, etc. weeks gestational age). The number of multiple births in our study population was small (N=284) and therefore may have limited the statistical power to detect a significant association between twins and neonatal jaundice, if one exists.

While exclusive breastfeeding has historically been an important predictor for jaundice, the mechanism behind the association is not well understood. Human milk inhibits the conjugation of bilirubin(32) with infants who are breastfed having 3 times the risk of bilirubin levels >12 mg/dl(42). The interaction between exclusive breastfeeding and difficulty feeding or reduced caloric intake has been discussed extensively in the literature as a potential biological mechanism explaining exclusive breastfeeding as a primary risk factor for hyperbilirubinemia. Weight loss during the neonatal period is another risk factor for jaundice(39). Low caloric intake results in increased hepatic circulation of bilirubin and is often observed the first few days before the mother's milk comes in(43). We did observe a significant interaction between exclusive breastfeeding and difficulty feeding. Exclusively breastfed infants with reported difficulty feeding were significantly more likely to be referred for jaundice, whereas among infants who did not have difficulty feeding, exclusive breastfeeding was protective [RR=0.72 (95%CI: 0.58-0.88); data not shown]. We did not include time to initiation of breastfeeding in multivariable analyses due to the context of the study and observed collinearity. A previous study that evaluated breastfeeding patterns in this

same study population observed that infants breastfed within 24 hours of birth were more than 8 times more likely to be exclusively breastfed(25).

Unique associations included the strong relationship between mustard oil massage given to the infant in the first two weeks of life and jaundice. This may be due to this population's observed practice of massaging babies outdoors where jaundice is more noticeable in direct sunlight. The application of mustard oil to a newborn's skin is an almost universal practice in this setting and is primarily considered by caretakers to improve the health of the infant and their skin (44). Alternatively, the observed increased risk of jaundice with increasing ambient temperature may be explained by the family's reluctance to be outdoors in the hot season due to extreme heat, thus reducing the potential for phototherapy. We also observed a strong association between *pahadi* ethnicity and jaundice in our study population. Infants in *madeshi* households were also significantly less likely to be exclusively breastfed, early initiators, or given colostrum compared to *pahadi* infants(25), all significant risk factors for jaundice in this analysis. However, in analyses stratified by these risk factors, the association between ethnicity remained unchanged, indicating the ethnicity difference is not fully explained by breastfeeding practices. People of *madeshi* ethnicity tend to have darker skin, and therefore it may be more difficult to observe jaundice in these infants, resulting in lower referral rates. The biological mechanisms behind these uniquely observed associations should be explored further.

In summary, the risk factors for neonatal jaundice supported by our study population comprise several known factors, namely sex, birthweight, breastfeeding patterns, primiparity, and difficulty feeding. Unique relationships observed included warmer air temperatures, oil massage, and ethnicity. Our study may help to explain the well-studied association between exclusive breastfeeding and neonatal jaundice by providing further evidence of an interaction with difficulty feeding and exclusive breastfeeding. These findings may be important in identifying those infants in a community setting without the benefit of laboratory tests that may require treatment for neonatal jaundice and thus reduce the morbidity and mortality associated with this condition.

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Corresponding author: James Tielsch, Department of Global Health, School of Public Health and Health Services, George Washington University, 2175 K Street, NW, Suite 200, Washington, DC 20037, USA. Phone +1-202-994-4124, Fax +1-202-994-1955, Email: jtielsch@gwu.edu

Figure 1. Cumulative distribution of age at visit (days) of incident referral for “yellow body/eyes”, Sarlahi, Nepal, 2003-2005.

Table 1. Bivariate Analysis of potential risk factors for incident neonatal jaundice, Sarlahi, Nepal, 2003-2005

	Referred for Jaundice	Total	Incidence Rate (per 1000 live births)	Adjusted RR (95% CI)*	p val- ue
Infant characteristic					
Sex					
Female	192	9137	21.0	1 (Ref)	<0.001
Male	364	9848	37.0	1.76 (1.48, 2.09)	
Birthweight (g)					
<2000	33	963	34.3	1.24 (0.86, 1.77)	0.24
2000-2499	121	4629	26.1	0.94 (0.76, 1.17)	0.59
2500-2999	230	8290	27.7	1 (Ref)	<0.01
≥3000	169	4523	37.4	1.35 (1.11, 1.64)	
Gestational age (wk)					
≥ 37 weeks	473	15518	30.5	1 (Ref)	0.08
34-37 weeks	74	3040	24.3	0.8 (0.63, 1.02)	
<34 weeks	9	414	21.7	0.72 (0.37, 1.37)	
SGA					
Not SGA	254	8103	31.3	1 (Ref)	0.34
SGA	299	10290	29.1	0.92 (0.78, 1.09)	
Intrapartum and maternal characteristics					
Exclusively breastfed					
Yes	172	5010	34.3	1 (Ref)	0.031
No	376	13302	28.3	0.82 (0.69, 0.98)	
Difficulty feeding at anytime over follow-up (28 days)					
No	402	16120	24.9	1 (Ref)	<0.001
Yes	154	2309	66.7	2.57 (2.14, 3.08)	
Breastfeeding - time to initia- tion					
<24 hours	427	10596	40.3	1 (Ref)	<0.001
≥ 24 hours	121	7716	15.7	0.39 (0.32, 0.48)	
Colostrum					
Not given	80	3387	23.6	1 (Ref)	0.027
Given	461	14977	30.8	1.30 (1.03, 1.65)	
Place of delivery					
Non-facility	389	16284	23.9	1 (Ref)	<0.001
Facility	145	1754	82.7	3.46 (2.88, 4.16)	
Skilled birth attendant at deli-					

	Referred for Jaundice	Total	Incidence Rate (per 1000 live births)	Adjusted RR (95% CI)*	p val- ue
very?					
No	382	16201	23.6	1 (Ref)	
Yes	147	1774	82.9	3.51 (2.92, 4.22)	<0.001
Color at birth					
Red all over	431	16323	26.4	1 (Ref)	
Red, except for extremities	12	305	39.3	1.49 (0.85, 2.62)	0.163
Blue/grey	41	587	69.8	2.64 (1.94, 3.6)	<0.001
Head abnormality at birth					
No	533	17942	29.7	1 (Ref)	
Yes	4	99	40.4	1.36 (0.52, 3.56)	0.533
Injury at birth					
No	503	17676	28.5	1 (Ref)	
Yes	17	188	90.4	3.16 (1.99, 5.01)	<0.001
Mustard oil applied in 1st 2 weeks of life					
No	12	39	307.7	1 (Ref)	
Yes	531	18365	28.9	0.10 (0.06, 0.15)	<0.001
Season of birth					
Cold (Dec-Feb)	88	4020	21.9	1 (Ref)	
Moderate (Mar, Apr, Oct, Nov)	232	8524	27.2	1.23 (0.96, 1.57)	0.097
Hot (May-Sept)	236	6441	36.6	1.65 (1.30, 2.11)	<0.001
Season of birth					
Cold (Nov-Feb)	133	6061	21.9	1 (Ref)	
Hot (Mar-Oct))	423	12924	32.7	1.48 (1.22, 1.79)	<0.001
Minimum ambient air temper- ature				1.03 (1.02, 1.05)	<0.001
Maternal age					
< 20 years	181	4924	36.8	1 (Ref)	
20-24.9 years	230	7535	30.5	0.83 (0.68, 1)	0.055
25-29.9 years	94	4091	23.0	0.63 (0.49, 0.8)	<0.001
30-34.4 years	36	1695	21.2	0.58 (0.41, 0.82)	0.002
≥35 years	15	740	20.3	0.55 (0.33, 0.92)	0.024
Parity					
0 previous live born	247	4984	49.6	1 (Ref)	
≥1 previous live born	309	14001	22.1	0.44 (0.38, 0.52)	<0.001
Multiple pregnancy					
Singleton	551	18,667	29.5	1 (Ref)	
Multiple birth	5	284	17.6	0.61 (0.26, 1.46)	0.269
Vaginal bleeding 7 days prior to delivery					

	Referred for Jaundice	Total	Incidence Rate (per 1000 live births)	Adjusted RR (95% CI)*	p val- ue
No	497	17262	28.8	1 (Ref)	
Yes	35	753	46.5	1.61 (1.15, 2.25)	0.005
Convulsions 7 days prior to de- livery					
No	532	17965	29.6	1 (Ref)	
Yes	2	53	37.7	1.28 (0.33, 4.99)	0.724
Fever 7 days prior to delivery					
No	513	17379	29.5	1 (Ref)	
Yes	21	655	32.1	1.09 (0.71, 1.68)	0.692
Prolonged Labor					
Labor<24 hour primiparous or <12 hour multiparous	330	12963	25.5	1 (Ref)	
Labor ≥24 hr primiparous or ≥12 hr multiparous	226	6022	37.5	1.47 (1.25, 1.74)	<0.001
HOUSEHOLD and SES					
Paternal literacy					
No	133	8116	16.4	1 (Ref)	
Yes	423	10,850	39.0	2.37 (1.96, 2.88)	<0.001
Maternal literacy					
No	258	13997	18.4	1 (Ref)	
Yes	298	4981	59.8	3.25 (2.76, 3.82)	<0.001
Paternal Education					
None	148	8625	17.2	1 (Ref)	
1-3 years	17	807	21.1	1.22 (0.74, 2.01)	0.428
4-6 years	80	2885	27.7	1.61 (1.23, 2.11)	0.001
7-9 years	91	2872	31.7	1.84 (1.42, 2.38)	<0.001
10+ years	220	3796	58.0	3.38 (2.75, 4.14)	<0.001
Maternal education					
None	268	14,327	18.7	1 (Ref)	
1-3 years	10	428	23.4	1.26 (0.67, 2.35)	0.473
4-6 years	61	1441	42.3	2.25 (1.72, 2.96)	<0.001
7-9 years	80	1235	64.8	3.46 (2.72, 4.42)	<0.001
10+ years	137	1554	88.2	4.71 (3.86, 5.75)	<0.001
Paternal occupation					
Does not work outside the home	6	176	34.1	1 (Ref)	
Farmer/unskilled laborer	341	13588	25.1	0.74 (0.33, 1.63)	0.451
Skilled laborer	209	5187	40.3	1.18 (0.53, 2.63)	0.68
Maternal occupation					
Does not work outside the	459	15979	28.7	1 (Ref)	

	Referred for Jaundice	Total	Incidence Rate (per 1000 live births)	Adjusted RR (95% CI)*	p val- ue
home					
Farmer/unskilled laborer	82	2728	30.1	1.05 (0.83, 1.32)	0.697
Skilled laborer	15	253	59.3	2.07 (1.26, 3.41)	0.004
Electricity					
No	329	14041	23.4	1 (Ref)	
Yes	209	4604	45.4	1.94 (1.63, 2.30)	<0.001
Television					
No	376	15232	24.7	1 (Ref)	
Yes	161	3406	47.3	1.91 (1.60, 2,29)	<0.001
Latrine					
Brick and cement	133	1676	79.4	1 (Ref)	
Pit latrine	34	543	62.6	0.79 (0.55, 1.14)	0.206
No latrine	371	16388	22.6	0.28 (0.24, 0.35)	<0.001
Ethnic group					
Hills (Pahadi)	350	5277	66.3	1 (Ref)	
Plains (Madeshi)	188	13357	14.1	0.21 (0.18, 0.25)	<0.001

*All analyses are adjusted for washing treatment group and account for the cluster-randomized design of the treatment allocation.

Table 2. Multivariable models of potential risk factors for incident neonatal jaundice, Sarlahi, Nepal, 2003-2005.

Risk Factor	Model 1 (n=18,393)	Model 2 (n=16,144)	Model 3 (n=15,758)
	Relative Risk	Relative Risk	Relative Risk
	95%CI	95%CI	95%CI
Infant characteristics			
Sex			
Female	1 (Ref)	1 (Ref)	1 (Ref)
Male	1.73 (1.45, 2.06)	1.61 (1.33, 1.95)	1.67 (1.38, 2.02)
Birthweight (g)			
<2000	1.33 (0.92, 1.93)	1.49 (1, 2.22)	2.01 (1.34, 3.01)
2000-2499	0.98 (0.78, 1.23)	1.06 (0.82, 1.36)	1.29 (1, 1.66)
2500-2999	1 (Ref)	1 (Ref)	1 (Ref)
≥3000	1.30 (0.99, 1.72)	1.38 (1.01, 1.88)	1.14 (0.83, 1.56)
Gestational age (wk)			
≥ 37 weeks	1 (Ref)	1 (Ref)	1 (Ref)
34-37 weeks	0.82 (0.63, 1.08)	0.95 (0.71, 1.28)	1.14 (0.85, 1.53)
<34 weeks	0.84 (0.42, 1.68)	0.90 (0.43, 1.88)	1.07 (0.52, 2.22)
SGA			
Not SGA	1 (Ref)	1 (Ref)	1 (Ref)
SGA	1.04 (0.79, 1.38)	0.95 (0.7, 1.3)	0.86 (0.63, 1.18)
Intrapartum and Maternal Characteristics			

Exclusively breastfed	Yes	1 (Ref)		1 (Ref)	(0.98, 1.59)
	No	0.77	(0.61, 0.96)	1.24	
Difficulty feeding at anytime over follow-up (28 days)	No	1 (Ref)		1 (Ref)	(1.09, 2.34)
	Yes	1.61	(1.1, 2.37)	1.60	
Colostrum	Not given	1 (Ref)		1 (Ref)	(0.64, 1.08)
	Given	1.12	(0.87, 1.44)	0.83	
Place of delivery	Non-facility	1 (Ref)		1 (Ref)	(0.56, 1.43)
	Facility	1.07	(0.61, 1.85)	0.90	
Skilled birth attendant at delivery?	No	1 (Ref)		1 (Ref)	(0.81, 2.03)
	Yes	1.85	(1.08, 3.19)	1.28	
Color at birth	Red all over	1 (Ref)		1 (Ref)	(0.92, 2.85)
	Red, except for extremities	1.50	(0.85, 2.65)	1.62	
	Blue/grey	1.66	(1.16, 2.39)	1.40	(0.98, 1.99)
Injury at birth	No	1 (Ref)		1 (Ref)	(0.84, 1.54)
	Yes	1.52	(0.8, 2.88)	1.54	

2.81)

Mustard oil applied in 1st 2 weeks of life

No

1 (Ref)

1 (Ref)

Yes

(0.09, 0.38)

0.24

(0.11, 0.5)

Minimum ambient air temperature

1.04

(1.03, 1.06)

1.04

(1.03, 1.06)

Maternal age

< 20 years

1 (Ref)

1 (Ref)

(0.97, 1.56)

1.02

(0.8, 1.29)

20-24.9 years

1.23

(0.97, 1.56)

1.02

(0.8, 1.29)

25-29.9 years

1.08

(0.78, 1.5)

1.02

(0.73, 1.42)

30-34.4 years

1.03

(0.67, 1.58)

1.08

(0.7, 1.66)

>=35 years

1.03

(0.56, 1.89)

1.03

(0.56, 1.9)

Parity

0 previous live born

1 (Ref)

1 (Ref)

(0.51, 0.84)

≥1 previous live born

0.52

(0.4, 0.66)

0.66

(0.51, 0.84)

Multiple pregnancy

Singleton

1 (Ref)

1 (Ref)

(0.07, 1.25)

Multiple birth

0.32

(0.08, 1.35)

0.30

(0.07, 1.25)

Vaginal bleeding 7 days prior to delivery

No

1 (Ref)

1 (Ref)

(0.77, 1.14)

Yes

1.31

(0.9, 1.93)

1.14

(0.77, 1.14)

Convulsions 7 days prior to delivery					1.69)
No	1 (Ref)	1 (Ref)			
Yes	0.77	(0.1, 5.73)	0.82	(0.12, 5.74)	
Fever 7 days prior to delivery					
No	1 (Ref)		1 (Ref)		
Yes	0.93	(0.56, 1.53)	1.09	(0.66, 1.8)	
Prolonged Labor					
Labor<24 hour					
primiparous or <12 hour multiparous	1 (Ref)		1 (Ref)		
Labor ≥24 hr primiparous or ≥12 hr multiparous	1.24	(1.02, 1.51)	1.25	(1.03, 1.51)	
Household and Socioeconomic Characteristics					
Paternal Education					
None			1 (Ref)	(0.65, 1.76)	
1-3 years			1.07	(0.71, 1.31)	
4-6 years			0.96	(0.66, 1.24)	
7-9 years			0.90	(0.81, 1.5)	
10+ years			1.10		
Maternal education					
None			1 (Ref)		
1-3 years			0.86	(0.46, 1.6)	

4-6 years	1.31	(0.95, 1.8)
7-9 years	1.38	(0.98, 1.93)
10+ years	1.28	(0.9, 1.83)
Paternal occupation		
Does not work outside the home	1 (Ref)	
Farmer/unskilled laborer	1.61	(0.62, 4.15)
Skilled laborer	1.72	(0.66, 4.44)
Maternal occupation		
Does not work outside the home	1 (Ref)	
Farmer/unskilled laborer	0.88	(0.68, 1.14)
Skilled laborer	0.87	(0.48, 1.57)
Electricity		
No	1 (Ref)	
Yes	1.19	(0.94, 1.5)
Television		
No	1 (Ref)	
Yes	0.88	(0.69, 1.12)
Latrine		
Brick and cement	1 (Ref)	
Pit latrine	0.62	(0.41, 0.95)
No latrine	0.57	(0.43, 0.74)

Ethnic group

Hills (Pahadi)

1 (Ref)

(0.21,
0.35)

Plains (Madeshi)

0.27

*All analyses are adjusted for washing treatment group, the interaction between exclusive breastfeeding and difficulty feeding, and account for the cluster-randomized design of the treatment allocation.

Table 3. Nested case-cohort analysis of maternal report of infant’s difficulty sucking or feeding during follow-up (28 days) and the association with incident neonatal jaundice, Sarlahi, Nepal, 2003-2005.

Exposure	Cases	Controls	Odds Ratio*	95% Confidence Interval
No report of difficulty sucking or feeding	515	5481	1	
Difficulty sucking or feeding	41	79	5.62	3.80, 8.32

* adjusted for washing treatment group and accounts for the cluster-randomized design of the treatment allocation.