Bundling, a Newly Identified Risk Factor for Neonatal Tetanus: Implications for Global Control

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Background. Bundling, which consists of wrapping an infant for prolonged periods in a sheepskin cover after dried cow dung is applied, is a common and apparently unique practice limited to the rural, mountainous regions of Northern Pakistan. The practice is initiated at various ages during the neonatal period. Its potential contribution to neonatal tetanus (NNT) had not been evaluated.

Methods. A population-based, matched, case-control study was undertaken to assess bundling and other factors potentially related to NNT in rural parts of the Northern Areas, Pakistan.

Results. Bundling instituted within the first 3 days of life was a substantial risk factor for NNT (odds ratio [OR] = 2.5, 95% confidence interval [CI]: 1.3-4.9). Other factors found risky for NNT were delivery on a straw surface and pre-delivery intravaginal application of ghee to the mothers. Handwashing by the delivery attendant and use of a new razor to cut the umbilical cord were protective.

Conclusions. Bundling is a significant risk factor for NNT in the mountainous regions of Northern Pakistan. While this practice is seemingly limited to these remote areas, the findings have broad implications since they indicate that NNT can derive from exposures of the umbilical wound at any time during the first several days of life. Thus, clean cord care at delivery is not itself sufficient to prevent NNT and control programmes need to address post-delivery sources of NNT.

Keywords: bundling, neonatal, tetanus, risks, sources

The Task Force for Child Survival and Development and the Global 2000 of The Carter Center, with cooperation from WHO, UNICEF, and the government of Pakistan, conducted a population-based survey in rural parts of the Northern Areas of Pakistan to define characteristics of neonatal tetanus (NNT) in this area and to assist in developing control efforts. Similar previous studies in other areas of Pakistan had identified several birth and post-birth cord care practices related to NNT.1-3

‘Bundling’ is a common and potentially hazardous neonatal practice that is unique to the Northern Areas. The practice consists of wrapping the trunk and lower extremities of infants in inverted sheepskin (fleece in contact with the baby’s body) for prolonged periods. Various substances, almost invariably including dried cow dung, are applied each time the infant is wrapped in the sheepskin. Bundling may begin at any time throughout the neonatal period. The abdomen and lower extremities are typically not covered with clothing beneath the sheepskin. The sheepskin is removed, excreta evacuated, and re-applied usually after intervals of one week or more.

Bundling has similarities to the traditional Chitrali practice of ‘swaddling’ whereby dried cow dung is applied from the baby’s waist to the ankles, the knees are drawn up and the baby is wrapped in a piece of cloth that is tied so as to keep the dung next to the body.4 The dung is applied from right after birth until the baby is 3 or 4 months old, and is left on for up to 12 hours before being changed. Cow dung is believed to confer warmth and strength to the infant. The relationship of swaddling to NNT was not quantified.

STUDY DESIGN
To assess potential risk factors for NNT, a population-based, matched case-control study was conducted in rural parts of the Northern Areas of Pakistan. A cluster
The questionnaire was designed to provide demographic information, tetanus toxoid immunization details, and a description of delivery and perinatal practices for all live births. Detailed questions were included about bundling and substances applied to the umbilical and circumcision wounds. Information about bundling included whether the infant was bundled, when bundling began, and the length of time the infant was bundled. Additional detailed information was collected about the application of ghee (clarified butter) to the umbilical wound, which had been identified as a risk factor in other studies. Pre-delivery application of ghee to mothers was also assessed.

For each live birth who died in the neonatal period, an additional form was completed that included details about the infant’s illness, including signs and symptoms of NNT. Information was also obtained on the age at onset of illness and age at death. Cases were considered to be NNT in the case-control study if their fatal illnesses began during the neonatal period and included both muscle rigidity and spasms.

Five controls were matched to each NNT case by sex, cluster and date of birth. Criteria for the selection of the control infants included survival of the infant throughout the neonatal period, and being born to a mother who had no history of receiving tetanus toxoid before delivery. Details of the rationale for these selection criteria are presented elsewhere.

Data Tabulation and Analysis
Data was originally tabulated and edited in DbaseIII+ by the members of Global 2000’s Islamabad-based staff. The dataset was converted to Epi Info 6.0 for analysis. The Dbase and Epi Info datasets were compared and discrepancies resolved, referring to the original questionnaires when necessary.

Applications to the umbilical wound were identified as ‘initial’ or ‘subsequent’. Initial applications were defined as those that were applied immediately after cutting the cord, while subsequent applications were applied post-delivery.

Questions with multiple responses were recoded to dichotomous variables for analysis. Matched odds ratios (OR) for variables and their associated probabilities based on Wald and likelihood ratio statistics were computed using a program for conditional logistic regression designed for use with Epi-Info data. Only matched OR are presented. In the stratified analysis, crude OR for bundling and bundling within the first 3 days were calculated and compared with Mantel-Haenszel summary OR in the presence of other variables to identify potential confounders. Woolf’s χ² for the heterogeneity of OR was calculated to assess interaction between the variables. Pearson correlation coefficients were calculated using SAS for selected variables that appeared significant in the matched analysis.

RESULTS
Overall Survey Findings
Only 11.1% of mothers of the 5232 live births in the survey gave histories of being appropriately immunized with tetanus toxoid when their child was born, defined as ≥2 doses within 5 years, with the second dose at least one month before delivery. The incidence of NNT was 11/1000 live births.

Overall Case-Control Findings
Information was tabulated for 354 live births, consisting of 59 NNT cases and 295 matched controls with a 1:5 match. The mean interval between the birth of a case and that of the matched control was only 2.4 days, with about 80% of the cases and their matched controls being matched on birthdates within 2 weeks of each other. Similar recall challenges were thus posed for mothers of cases and their matched control mothers.

Nearly all cases were normal at birth (95%) and stopped sucking at onset of illness (95%). The average age at onset was 7.1 days. Only 12 cases were seen and diagnosed by a physician. When shown a photograph of a newborn with typical physical manifestations of NNT, all but two of the mothers of cases indicated that the illnesses in their newborns were similar. Of the 59 cases, 44.1% were females and 55.9% were males.

The mean age of delivering mothers was 27 years old, and did not differ significantly for case and control mothers. This was the first child for only 16.2% of the delivering mothers. Among mothers who had prior live births, the prior live birth of case mothers was far more likely to have died, 20.5% for cases and 9.7% for controls (P = 0.045 by χ²). One of the 295 control mothers...
and two of the 59 case mothers identified at least one of their prior live births as having developed NNT (P = 0.07 by Fisher's Exact [2-tail]).

**Specific Delivery and Cord-Care Practices**

The frequencies of delivery practices for cases and controls are summarized in Tables 1 and 2, along with their OR.

There were no significant differences in the type of attendant at birth. The vast majority of cases and controls were delivered by relatives, and about a quarter of all deliveries in these remote, difficult-to-access areas were self-deliveries.

The risk of NNT was significantly increased by delivery on a straw surface (P = 0.002 by Wald statistic), the most common delivery surface for cases, and significantly reduced by delivery on a bamboo mat (P = 0.015 by Wald statistic). No other delivery surfaces were significantly related to NNT risk. The numbers of cases and controls delivered on a surface of dried cow dung were too few to permit a reliable assessment.

Handwashing by the delivery attendant was protective (P = 0.02 by Wald statistic). Pre-delivery applications of ghee (clarified butter) to the mother's vagina, abdomen, or perineum was substantially more common for case than control mothers. However, these three uses of ghee nearly always occurred together. Contamination of the newborn with ghee may be more likely when the ghee is applied intra-vaginally. Thus, only vaginal ghee exposure (P = 0.01 by Wald statistic) was used in subsequent analyses.

Instruments used to sever the umbilical cord differed in frequency among cases and controls. A new razor was protective (P = 0.004 by Wald statistic), while old razors had borderline hazard (P = 0.06 by Wald statistic). No other implements significantly influenced risk, including use of a kitchen knife.

Substances were applied to the umbilical wound of cases and controls in similar frequencies at delivery and post-delivery (Table 2). No substances, including ghee, were significantly related to NNT. The number of infants exposed to antibiotics, animal dung, ash and disinfectants were too few to permit a confident appraisal of risk. Dry cord care, ‘nothing’, did not confer protection.

**Circumcision**

Of the 198 male infants, 75 were circumcised, including 11 (34%) of the cases and 64 (40%) of the controls. Circumcision was not significant in the matched analysis. All 11 circumcised boy cases were at least one week old at the time of circumcision, and 10 were bundled for ≥3 days immediately after circumcision. Further, the age at which bundling started for boy cases differed in accord with their circumcision status. Circumcised cases began bundling on average at 5.8 days of age, while for uncircumcised boy cases bundling began on average at 2 days old (P = 0.002 by Kruskal-Wallis).

A likely ‘rounding’ bias was noted for age at circumcision, with 54 of 64 circumcised controls and 10 of 11 cases being reported to have been circumcised at exactly 7 days old. This, and the small number of circumcised cases, compromised the reliability of comparing the frequency of circumcision before onset of illness in cases with the frequency with which controls were circumcised before the age at onset of their matched cases (OR = 3.0, P = 0.59 by Wald statistic).
### Table 2: Substances applied to the umbilical wound

<table>
<thead>
<tr>
<th>Substance</th>
<th>At delivery</th>
<th></th>
<th></th>
<th>Post-delivery</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency among</td>
<td>Matched odds ratio (95% confidence interval)</td>
<td>Frequency among</td>
<td>Matched odds ratio (95% confidence interval)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Cases Controls</td>
<td></td>
<td></td>
<td>Cases Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oil</td>
<td>56% 55%</td>
<td>1.2 (0.31-4.4)</td>
<td></td>
<td>59% 59%</td>
<td>1.0 (0.22-4.6)</td>
<td></td>
</tr>
<tr>
<td>Ghee</td>
<td>19% 16%</td>
<td>1.4 (0.54-3.8)</td>
<td></td>
<td>15% 20%</td>
<td>0.6 (0.25-1.6)</td>
<td></td>
</tr>
<tr>
<td>Nothing</td>
<td>8% 9%</td>
<td>0.8 (0.26-2.7)</td>
<td></td>
<td>10% 9%</td>
<td>1.1 (0.40-3.0)</td>
<td></td>
</tr>
<tr>
<td>Surma</td>
<td>5% 9%</td>
<td>0.4 (0.10-1.8)</td>
<td></td>
<td>5% 4%</td>
<td>1.2 (0.30-4.6)</td>
<td></td>
</tr>
<tr>
<td>Turmeric</td>
<td>5% 5%</td>
<td>1.1 (0.27-4.4)</td>
<td></td>
<td>3% 8%</td>
<td>0.4 (0.08-1.5)</td>
<td></td>
</tr>
<tr>
<td>Grasses</td>
<td>5% 6%</td>
<td>0.4 (0.04-4.3)</td>
<td></td>
<td>7% 6%</td>
<td>1.8 (0.19-16.1)</td>
<td></td>
</tr>
<tr>
<td>Antibiotic</td>
<td>3% 1%</td>
<td>3.3 (0.56-20.0)</td>
<td></td>
<td>3% 1%</td>
<td>5.0 (0.70-35.5)</td>
<td></td>
</tr>
<tr>
<td>Nuts</td>
<td>2% 1%</td>
<td>2.5 (0.23-27.6)</td>
<td></td>
<td>7% 6%</td>
<td>1.8 (0.19-16.1)</td>
<td></td>
</tr>
<tr>
<td>Cow dung</td>
<td>2% 1%</td>
<td>2.5 (0.23-27.6)</td>
<td></td>
<td>0% 1%</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>0% 0.3%</td>
<td>–</td>
<td></td>
<td>0% 0.3%</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Disinfectant</td>
<td>0% 2%</td>
<td>–</td>
<td></td>
<td>2% 1%</td>
<td>2.0 (0.14-29.3)</td>
<td></td>
</tr>
<tr>
<td>Camel dung</td>
<td>0% 0%</td>
<td>–</td>
<td></td>
<td>0% 0.3%</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Although circumcision could not be directly incriminated as a risk factor, application of cow dung directly to the circumcision wound was found to increase risk of NNT (OR = 25.8, P = 0.002 by Wald statistic). Direct application of cow dung to circumcision wounds was also strongly associated with bundling (P = 0.019 by $\chi^2$).

**Bundling**

Overall, approximately 55% of both cases and controls were bundled after delivery. Bundling began anywhere from the first day to the tenth day of life for cases, and throughout the neonatal period for controls. Cases were bundled for an average of about 12 days and controls were bundled on average about 102 days (P < 10^{-6} by Kruskal-Wallis). This reflects the greater number of days control infants were 'at risk' for this practice during and after the neonatal period compared with cases, who all became ill and died. When bundling was analysed for each day of life in the first week after delivery, the day-specific matched OR were 3.17 (95% CI: 1.0-10.0), 1.75 (0.61-5.0), 2.53 (1.0-6.2), undefined, 0.59 (0.07-5.1), undefined, and 1.05 (0.33-3.3), respectively, among infants who were alive and not previously bundled on that day of age. The day 1 and day 3 OR were statistically significant (P = 0.049 and 0.043, respectively, by Wald statistic). No cases were bundled on days 4 and 6. Bundling within the first 3 days of life was significantly more likely among cases (36%) than controls (20%), and subsequent multivariate analyses employed this variable (OR = 2.5, P = 0.006 by Wald statistic). Among babies alive and not previously bundled on the fourth day of life, a similar proportion of cases, 11/36 (30.6%), and controls, 75/238 (31.5%), were bundled from the fourth through seventh day of life (P = 0.94 by $\chi^2$). This suggests that risks of umbilical wound contamination may be mainly confined to the first 3 days of life.

The risk of bundling was also explored after determining whether matched controls had been exposed to bundling before the age at which their matched case died, thus 'censoring' out bundling exposures of controls who were bundled after their matched case had died. Overall, the risk associated with exposure to bundling before the death of cases was not significantly associated with NNT. However, limiting the analysis to those exposures occurring among cases who died within the first week of life and their matched controls revealed a highly significant risk for NNT (OR = 15.4, P = 0.002 by Wald statistic).

Stratified analysis of bundling within the first 3 days after delivery revealed no significant interaction effects with other variables, using Woolf's test for heterogeneity of OR. In addition, none of the other variables were found to be significant confounders.

**Multivariate Analysis**

Conditional logistic regression models, including all variables found significant in conditional univariate tests, were fitted to the data to better estimate the effect of bundling within the first 3 days of life on the risk of NNT. Despite significant correlations among several variables, five variables remained significantly associated with NNT (Table 3), and each contributed significantly to the model. Addition of the variable for cow dung...
dung applied directly to the circumcision wound resulted in failure of the model to converge. Neither delivery on a bamboo mat, use of an old razor to cut the cord, nor umbilical ghee applications contributed significantly when added to this model.

DISCUSSION
The increased likelihood of NNT among previous live births to case mothers, while of borderline significance ($P = 0.07$) in the present study, is consistent with increased frequencies seen in our earlier studies. In all four studies, case mothers were more likely to have had a death occur among prior live births. These observations may be indicative of delivery practices associated with the prior delivery which were repeated for the present delivery. These variables were not included in multivariate analysis because information was not available for the cause or time of death, and they were highly associated with other variables that were deemed likely to be proximate causes. Control mothers having a prior live birth alive at the time of the present delivery were less likely than control mothers with a prior death to bundle within the first 3 days ($P < 0.01$ by $\chi^2$), and more likely to have a birth attendant who washed their hands ($P = 0.028$ by $\chi^2$).

Application of ghee before delivery to the mother’s vagina, perineum and abdomen were very highly correlated with each other, thus precluding a determination of the independent effect for each. Pre-delivery applications of ghee to mothers is a one-time event, and the mechanisms of contamination with tetanus spores are currently undetermined. Maternal ghee practices became disclosed after our earlier studies were concluded.

Delivery on a straw surface was also found to be significantly associated with NNT. Animal dung is often used to fertilize fields from which straw is harvested; therefore straw may be more frequently or heavily contaminated with Clostridium tetani spores than other delivery surfaces. Such spores could contaminate the fresh umbilical wound either through direct contact or indirectly through the hands of the delivery attendants. Increased risk was not noted for delivery on a surface of hay, which might be expected to have spore contamination similar to straw; however, a hay delivery surface was infrequent among both cases and controls, and only three cases were so exposed.

Having a birth attendant who washed their hands prior to delivery was found to be significantly protective for NNT, confirming prior findings, and re-emphasizing the importance of handwashing as an infection control practice.

Even though the use of a new razor was found to be significantly protective in this study, the associated risk between the tool used to cut the umbilical cord and NNT has been refuted in prior studies. Therefore, use of a new razor may be serving as a marker for other protective practices, and use of a new razor itself may not be intrinsically less risky for NNT.

The inability to substantiate an association between ghee applications to the umbilical wound and NNT may be related to the limited frequency with which these practices were observed. In part, this is a consequence of the high frequency with which bundling began during the first few days of life. Once bundled, further applications of ghee and other substances to the umbilical wound were effectively curtailed. Indeed, post-delivery applications of ghee to the umbilical wound occurred in only 5 of 189 bundled infants, and only to 62 of 160 case and control infants who were not bundled. Further, the lack of repeated applications may be very important in explaining these results since the mechanism underlying risk for umbilical ghee appears to be cumulative contamination of ghee set aside in special containers for repeated sequential applications.

There is suggestive evidence from the present study that circumcision may increase the risk of NNT, particularly if hazardous exposures such as bundling occur at or soon after circumcision. Cow dung applications to the circumcision wound posed significant risks for NNT, even though circumcision per se could not be incriminated. The preponderance of male cases is also consistent with such a risk.

Bundling within the first 3 days after delivery, a time period throughout which nearly all cases and all controls were alive and at risk of exposure, was associated with an increased risk of NNT. Analyses suggested that
the risk of this hazardous practice was mainly limited to the first 3 days of life, thus also suggesting that risks of umbilical wound contamination leading to NNT may generally be mainly confined to the first several days of life. Such observations required age-specific data on bundling which, in turn, were available solely because we observed the outcome of an ‘experiment’ with bundling being initiated at different days. Such an experiment could not have been deliberately contrived and studied for ethical reasons. Bundling was also confirmed to be a risk factor when exposures of controls to bundling were censored in accord with the age at death of matched cases dying in the first week of life. The overall risk associated with bundling is biased by the large number of control infants who were bundled after the third day of life when the umbilical cord wound may have healed sufficiently to sharply reduce the risk of NNT consequent to spore contamination. The increased overall exposures of controls as a result of their increased survival is a ‘survival bias’ that may also obscure relationships. Without appropriate control of this bias during analysis, practices unrelated to NNT risk could potentially appear protective, and truly risky practices such as bundling and circumcision may not appear risky, or even appear protective. Previously reported protective effects of circumcision, and ear piercing appear likely to be examples of this bias.

If the OR for NNT and bundling in the first 3 days of life (2.5) is assumed to reflect relative risk, and if it is assumed that about one in five of all newborn infants are so exposed (the frequency in non-case infants who comprise nearly all live births), and given the observed NNT incidence of 11/1000 live births, then the total curtailment of this practice could reduce NNT deaths by about 23%.

Bundling-associated NNT cases may be controlled by improved coverage of women of child bearing ages with tetanus toxoid and educational programmes to curtail the use of animal dung, including programmes to substitute talcum powder for animal dung. The latter intervention has been successfully employed in the Chitrali area of Pakistan.

Our observations on bundling are consistent with earlier observations of the significant impact of substances solely applied to the umbilical wound during the first few days of life (but not concurrently applied at delivery), such as ghee and topical antibiotics. Further, it assists in explaining the occurrence of NNT in babies born in presumed aseptic hospital and other clean delivery situations. Simply adopting the ‘three cleans’ at the time of delivery (clean hands, clean delivery surface, clean cut) is not sufficient to prevent NNT in babies born to mothers who have not been appropriately immunized with tetanus toxoid.

Further studies are needed to determine the best methods of cord care during the first several days of life, with special emphasis on the possible protective effects of topical antimicrobials.

REFERENCES

(Revised version received December 1995)