Neonatal tetanus (NNT) currently kills about half a million babies a year, ranking second to measles as the top killer of children among the six vaccine preventable diseases, according to WHO. Neonatal tetanus usually results from the umbilical wound becoming contaminated with the spores of *Clostridium tetani*, the anaerobic Gram-positive bacilli, found in the soil and in high concentrations in horse and cattle droppings.

The ideal prevention of NNT is through the combined efforts of clean deliveries, clean cord care including the routine use of topical antimicrobials on the umbilical wound, and immunization of women with tetanus toxoid to assure transplacental transfer of antibodies protective against toxin produced by *Cl. tetani*. It is recommended that a previously non-immunized pregnant woman receives two intramuscular injections of tetanus toxoid (TT), 4–6 weeks apart at least 4 weeks before delivery. Topical antibiotics to circumcision wounds offer an additional preventive strategy in areas where neonatal circumcision is practised.

Neonatal tetanus has been nearly eliminated in developed countries, but it continues to persist in developing countries, especially in rural areas where many women are unreachable for vaccination and risk of exposure is high. Seventy-five per cent of the current global burden of NNT lies in the following countries: Bangladesh, China, India, Indonesia, Nigeria and Pakistan.

From 1989 to 1991, a series of case-control studies were conducted in Pakistan to assess risk factors for NNT. In three studies, initial (at delivery) and subsequent (after day of delivery) ghee applications to the umbilical wound were documented to be risky for NNT. Ghee, a clarified butter used for cooking and dressing purposes, has been an integral part of Indian and Pakistani culture and is believed to possess special powers of healing and strengthening. Home-made ghee is most highly valued. It

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Neonatal tetanus associated with topical umbilical ghee: covert role of cow dung

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**Background**

Previous studies in Pakistan have shown that ghee (clarified butter) is commonly applied to umbilical wounds of neonates, and have documented that such applications are a risk factor for neonatal tetanus (NNT). In-use contamination of ghee with *Clostridium tetani* has been demonstrated, but mechanisms underlying the risk of ghee have been incompletely evaluated epidemiologically.

**Methods**

Detailed information on ghee usage, including fuels used to heat it, was obtained from cases of NNT (n = 229) and their matched controls (n = 687) from a population-based study of NNT in Punjab Province, Pakistan. Design variables were created to evaluate the impact of different fuel sources on risk of ghee applications.

**Results**

Nearly one-third of all infants had ghee applied, and it was nearly always heated before application to umbilical wounds of newborns. After controlling for all factors found to be significantly associated with NNT in conditional logistic regression, only ghee that had always been heated with dried cow dung fuel was significantly associated with NNT. Topical antimicrobials and ghee were never applied together.

**Conclusions**

Ghee applications to umbilical wounds, when heated with ‘clean’ fuels, appear to pose no increased risk of NNT, although handling practices undoubtedly result in hazardous microbial contamination. In contrast, ghee heated with dung fuel was significantly associated with NNT. The effective promotion of topical antimicrobials might help reduce ghee use, since the intended purpose of each is to enhance healing.

**Keywords**

Neonatal, tetanus, ghee, umbilical, topical, prevention, case-control, antimicrobials

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is considered to signify affluence and is often presented to a pregnant woman as a gift near her time of delivery.5

Ghee as a risk factor was discovered in North West Frontier Province (NWFP) in the first community-based study of the Pakistan series. Ghee was applied to umbilical wounds of 57% cases and 33% controls (P = 0.01 by χ²). Notably, three of the four NNT cases delivered by a medically trained attendant in a hospital received ghee dressings after discharge. It was estimated that the total curtailment of this practice could reduce NNT death rates by 43%.6

In a hospital-based study in NWFP any ghee application was confirmed to be risky (odds ratio [OR] = 1.72, 95% CI : 1.03–2.87). Ghee applications to cases were more likely to be made from cows’ milk compared to controls (OR = 2.93, 95% CI : 1.69–5.08). Water buffalo as a source of milk for ghee was more frequent for controls than cases (25.8% versus 18.6%); ghee of vegetable origin was similar for both groups. On average, ghee was applied twice daily for both cases and controls.7

A second hospital-based study in Peshawar focused in detail on patterns of ghee use. Both initial (OR = 3.82, 95% CI : 1.62–9.0) and subsequent ghee (OR = 1.81, 95% CI : 1.08–3.05) were found to be significantly risky. Interviews with case and control mothers revealed that ghee was almost always transferred from a central household ghee pot to a separate smaller container that was repeatedly used as the source for topical umbilical applications. Ghee in the secondary container was almost always heated before each use. During visits to households of cases, mothers were observed during 16 simulations of its preparation and use. In addition to confirming the above practices, it was noted that mothers usually put fingers into the ghee to assure that it was cool enough to apply to the infant. One-fourth of the ghee heated in secondary containers, but none of the ghee in the central pots, were culture positive for Cl. tetani, demonstrating that contamination derived mostly from in-use practices. Dried cow dung was noted to be the fuel used to heat contaminated ghee in several instances.5

The present study, conducted in Punjab Province in 1990, further investigated the details of ghee use along with other known risk factors. This analysis focuses on the different fuels used to heat ghee before application to the infant and their associations with the risk of NNT.

Study Design
This population-based study was carried out in rural areas of Punjab Province by staff members from the Task Force for Child Survival and Development and Global 2000 in 1990. Other sponsors included WHO, UNICEF, and the government of Pakistan. A structured questionnaire was completed by trained student Lady Health Visitors who interviewed mothers of 23,670 live births occurring over a preceding year in 60 clusters. Information on demographics, pre-delivery practices, umbilical cord care, and circumcision (if performed) was obtained. Relevant to this analysis, interviewers determined whether ghee was applied to the infant’s umbilical wound at and/or after the first day of delivery. If subsequent ghee was used, they asked for the following information: whether it was transferred to a second container for use on the child; the total times ghee was heated for use on child; whether the person applying ghee washed hands after heating ghee; type of fuel used; how often the ghee was heated before use; whether ghee was home-made or commercial; the source of milk (if home-made); brand name (if commercial); any substances added before use; number of times a day and total days ghee was applied.

Detailed information was collected on signs and symptoms of all illnesses in infants who died in the neonatal period.

Case and control selection
The case definition included any infant born alive in rural Punjab who died within 30 days of birth, and had at least six of the eight following signs of NNT: normal at birth; suckled before becoming ill; had trouble opening mouth after onset of illness; had clenched hands; had dorsiflexed feet; had generalized spasms; had spasms initiated by touch; or had muscle rigidity. Three controls matched for each case were chosen among infants having survived the neonatal period and whose mothers had no history of TT vaccination. Matching of these infants, all of whom were born at home, was based on date of birth, sex and cluster. This analysis includes all 229 cases and their 687 matched controls. Prior publications have addressed subsets of these subjects in efforts to disclose risks of umbilical8 or circumcision sources.4 Factors found to be significant in these studies have been incorporated into the present analysis.

Data analysis
The initial file was created in Database III+, and converted to an EpiInfo 6.0 file for data analysis. The EpiInfo file itself was subsequently converted to a SAS file in order to use Statistical Analysis Software (SAS)10 for performing multivariate analysis and calculating Pearson correlation coefficients. The number of missing or ‘don’t know’ responses varied by questionnaire item. The results are based on the number of records with information for a given variable, not on the total number of subjects in the study.

The main exposure of interest was the type of fuel used to heat ghee. Four design variables were created for subsequent ghee heated by different types of fuel: (1) dung fuel only, (2) dung and wood (mixed fuel), (3) wood or gas only, and (4) unknown fuel. The referent group consisted of those who did not have subsequent ghee applied to their umbilical wounds. The four design variables were modelled in conditional logistic regression. Variables considered for inclusion in the models were those found to be significantly protective or risky in previous analyses: medically trained delivery attendant, dirty delivery surface, maternal ghee applied to perineum, abdomen, or intra-vaginally before delivery, hand washing by attendant, scissors to cut cord, ash or dung applied to umbilical wound, continuous use of antimicrobials applied to cord, circumcision before onset time, and antimicrobials on circumcision wound. Interactions with the design variables were assessed with the ‘chunk test’.11

Results
Ghee usage
Ghee had been applied at delivery to 211 infants, and 202 (96%) of these infants also had ghee applied subsequently. Thus, an independent assessment of the effects of initial ghee alone was not feasible. In 95 instances, ghee had only been applied subsequently. No information was collected on fuel used to heat ghee that was applied initially.
Overall, 32.5% of 913 subjects with known responses had subsequent ghee applied to the umbilical wound, including 84 cases and 213 controls (OR = 1.29, 95% CI: 0.93–1.8). Cows’ milk was the source of ghee for 23% of cases and 20% of controls (P = 0.64), with water buffalo milk accounting for all other sources. Among those with known fuel for heating ghee, 19.5% cases and 10.6% controls used dung as the only source of fuel (P = 0.076). Mixed fuel (wood and dung) was similar for both groups, 3.9% cases and 6.5% controls (P = 0.58). Other sources of fuel were wood or gas only, used for 76.6% cases and 82.9% controls (P = 0.30). Water buffalo dung was reported as a fuel in only two of the 52 instances where dung fuel was used to heat the ghee.

The intensity of exposure to subsequent ghee heated with only dung fuel was next examined. Analysis was restricted to ghee applied before onset of illness for cases or before time of onset in their matched cases for controls. This approach, which censored exposures of controls in accord with onset age of NNT in their matched cases, had proven useful in demonstrating risks of binding and circumcision. The average total times subsequent ghee had been heated with dung as the only source of fuel was higher for cases (1.7) than for controls (0.71) (P = 0.057 by Mann-Whitney U) in this analysis.

There was also a trend towards a protective effect among those applying ghee who reported washing hands after heating ghee (OR = 0.56, P = 0.10). Washing hands was not significantly protective in the subgroup who used only dung as fuel, although small numbers hampered this assessment.

The use of ghee made from cows’ milk and the use of cow dung as a fuel source were significantly correlated; when ghee prepared from cows’ milk was used, cow dung fuel was used to heat it 30% (16/53) of the time, whereas ghee made with water buffalo milk was heated with dung fuel only 15% (31/205) of the time (P = 0.01, r = 0.16 by Pearson’s).

Interestingly, neither initial nor subsequent ghee was applied to umbilical wounds in any of the 75 instances where antimicrobials were continuously applied.

**Multivariate analysis**

Variables found significant in previous analyses of this data set were controlled for in all models, including use of scissors to cut the cord, ‘dirty cord’ (dung or ash applied to the umbilical wound), ‘maternal ghee’ (pre-delivery application of ghee to abdomen, perineum or vagina of mother), design variables for use or non-use of antibiotics on circumcision wounds of boys circumcised before time of onset, with girls and the remaining boys used as their referent group, ‘continuous cord antimicrobials’ (antibiotics or antiseptics applied at and after the day of delivery), and ‘delivery on a dirty surface’ (any surface other than clean cloth, plastic sheet or bed). Both ‘hand washing’ and ‘medically trained attendant’ were left out of the models due to collinearity with variables that were included.

After controlling these variables, only the ‘dung fuel only’ design variable was associated with a significantly increased risk of NNT (Table 1), while the other three design variables neither increased nor decreased risk significantly. The OR for ghee use in the very large group who had only used ‘clean fuels’ was at unity. Somewhat surprisingly, the OR for ghee that was sometimes heated with dung fuel was not elevated. However, the number of observations in this group is small, and confidence bounds widely overlap 1.0. Available data do not permit a quantitative assessment of the frequency with which dung versus other fuel was used in this group.

Other variables found to have risky point estimates were ‘dung or ash applied to cord’ (OR = 3.5, 95% CI: 1.9–6.4), ‘maternal ghee’ (OR = 1.5, 95% CI: 0.99–2.25), and ‘circumcision without topical antibiotics’ (OR = 5.1, 95% CI: 1.8–14.4). As observed previously, the OR for circumcised boys treated with antibiotics (1.1) was very similar to their referent group (1.0) who had only umbilical wounds as presumed potential sources for NNT. The point estimate for continuous use of antimicrobials on umbilical wounds was protective (OR = 0.46, 95% CI: 0.18–1.2). ‘Subsequent ghee’, when substituted in place of the four design variables, resulted in an insignificant OR (1.17, 95% CI: 0.77–1.77). No significant interactions were noted between design and other variables.

**Discussion**

In this case-control study the overall use of subsequent ghee was not found to be significant, but the use of design variables was extremely helpful in elucidating the influence of fuel type on risk of ghee applications. Ghee’s lack of overall significance in this instance could be explained by the fact that the contamination of ghee appears to depend on the type of fuel used to heat it. These observations thus help resolve the mechanisms underlying the initial observations of ghee umbilical dressings as a risk factor for NNT.

The frequency of using cow dung as fuel to heat ghee is likely to have varied from study to study. As noted in an earlier study, possession of cattle offers access to both their milk and their dung. Although dung fuel was indeed used more frequently to heat ghee made from cows’ milk in that study, the difference was not statistically significant. The currently observed significant correlation between use of ghee prepared from cows’ milk and the use of cow dung fuel to heat it, provides further evidence of the likely mechanism behind the earlier reported increased risk from ghee made from cows’ milk, even though information on the type of fuel used to heat ghee was not collected in that study. It also suggests why overall ghee use may have been more readily identified as a risk factor in the earlier

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**Table 1** Impact of fuel used to heat ghee on risk of neonatal tetanus

<table>
<thead>
<tr>
<th>Subsequent ghee</th>
<th>No.</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Not applied to umbilical wound</td>
<td>616</td>
<td>1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>2 Applied to umbilical wound</td>
<td>297</td>
<td>1.2 (0.8–1.8)</td>
<td>0.462</td>
</tr>
<tr>
<td>(a) always heated with dung fuel</td>
<td>36</td>
<td>2.7 (1.1–6.6)</td>
<td>0.026</td>
</tr>
<tr>
<td>(b) sometimes heated with dung fuel</td>
<td>16</td>
<td>0.2 (0.02–1.7)</td>
<td>0.133</td>
</tr>
<tr>
<td>(c) never heated with dung fuel</td>
<td>224</td>
<td>1.0 (0.7–1.6)</td>
<td>0.90</td>
</tr>
<tr>
<td>(d) heated with unknown fuels or unheated</td>
<td>21</td>
<td>1.8 (0.6–5.6)</td>
<td>0.31</td>
</tr>
<tr>
<td>Total</td>
<td>297</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Conditional logistic regression results controlling for maternal ghee, dirty cord, topical antimicrobials on the umbilical wound, use of scissors to cut the cord, dirty delivery surface, and circumcisions with and without topical antibiotics on the circumcision wound.

<sup>b</sup> Referent group.
studies; about three-quarters and two-thirds of the subsequently used ghee in those studies was made from cows' milk, versus only one-fifth in the present study. Water buffalo milk predominated as the source for ghee here, and this ghee was significantly less likely to be heated with dung fuel.

This analysis provides epidemiological support for the earlier microbiological observations of ghee contamination after heating and manipulating. Ghee in itself may pose little risk until it becomes contaminated in-use with tetanus spores. Although the exact sequence was not documented in this study, the likely mechanism of introducing tetanus spores was contamination of the hands by tending the fire and then touching the ghee.

Mothers nearly always applied the ghee, and it was assumed, as documented in earlier simulations, that they also heated the ghee. Although no significant elevation of NNT risk was noted when clean fuel sources were used to heat ghee, the in-use practices described will still result in microbial contamination. While simply avoiding ghee use entirely would avoid these risks, a variety of social and cultural factors make such a recommendation unlikely to be effective.

In the present multivariate analysis, the continuous use of antimicrobials on umbilical wounds was not significantly protective. However, in a previous analysis where circumcised boys that are included in the present analysis were excluded, the variable was significantly protective. Boy cases who were excluded because they also had circumcision wounds as possible sources had a higher frequency of treatment with umbilical antibiotics (11%) than was found among the cases included in the previous analysis (3%). The present data set included these boy cases, thus weakening the findings for topical antimicrobials in reference to the other analysis. These observations confirm the need to exclude boys who had both umbilical and circumcision wounds as potential sources in order to clearly establish the role of topical umbilical agents. Topical umbilical agents would not prevent NNT that actually derived from circumcision wounds.

The effective promotion of topical umbilical antimicrobials might help to displace ghee use, since both are believed to enhance healing and these two substances were, in fact, never used together. Topical antimicrobials to umbilical and circumcision wounds and tetanus toxoid immunization of mothers are complementary strategies that can work effectively even if lapses in delivery and wound care occur. Optimal control of NNT requires that each preventive measure be actively promoted.

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