Breastfeeding and the Risk of Life-threatening Enterotoxigenic Escherichia coli Diarrhea in Bangladeshi Infants and Children

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ABSTRACT. Objective. To assess the relationship between breastfeeding and the risk of life-threatening enterotoxigenic Escherichia coli (ETEC) diarrhea among Bangladeshi infants and young children <36 months of age.

Design. Case–control study.

Setting. A rural Bangladesh community.

Participants. A total of 168 cases with clinically severe ETEC diarrhea detected in a treatment center-based surveillance system during 1985 to 1986 and 3679 controls selected in three surveys of the same community during the same calendar interval.

Outcomes. Cases and controls were compared for the frequency of antecedent breastfeeding patterns.

Results. Compared with other feeding modes, exclusive breastfeeding of infants was associated with significant protection against severe ETEC diarrhea (relative risk [RR] = 0.51; 95% confidence interval [CI]: 0.28,0.96). However, during the second and third years of life, the risk of this outcome was similar in both breastfed and nonbreastfed children (RR = 0.98; 95% CI: 0.45,2.12), and no significant overall protective association between breastfeeding and severe ETEC diarrhea was evident during the first 3 years of life (RR = 0.86; 95% CI: 0.43,1.74).

Conclusions. Exclusive breastfeeding appeared to protect infants against severe ETEC diarrhea, but breastfeeding was not associated with protection after infancy, nor was it associated with a major overall reduction of severe ETEC disease during the first 3 years of life. Although not diminishing the importance of breastfeeding, our findings suggest that other interventions, such as immunization and education about proper food hygiene, may also be required in efforts to prevent this major pediatric disease. Pediatrics 1997;100(6). URL: http://www.pediatrics.org/cgi/content/full/100/6/e2; enterotoxigenic Escherichia coli, breastfeeding, diarrhea.

ABBREVIATIONS. ETEC, enterotoxigenic Escherichia coli; LT, heat stable toxin; ST, heat stable toxin; RR, relative risk; CI, confidence interval.

METHODS

Setting for the Study

The study was conducted in the Matlab field studies area of the International Centre for Diarrhoeal Disease Research, Bangladesh, a rural area with approximately 200 000 residents. In conjunction with a field trial of oral cholera vaccines initiated in 1985,2 surveillance was instituted for all patients seeking care for diarrhea at the three Matlab diarrheal treatment centers. In this surveillance, patients received systematic clinical and microbiological assessments, and selected dietary and sociodemographic information was collected. Fecal specimens were evaluated for ETEC by testing five lactose-fermenting colonies for heat labile (LT) and heat stable lactose.
Selection and Definition of Cases

Cases were children who resided in Matlab and were detected as having an episode of clinically severe ETEC diarrhea diagnosed during 1985 and 1986 in one of the three Matlab treatment centers. Clinically severe disease was chosen as the target outcome because of its public health importance, and because limitation of the case group to episodes of severe ETEC diarrhea, for which solicitation of care could be presumed to be nondiscretionary, minimized the potential for selection biases related to differences in the use of the treatment centers by the Matlab population. To assemble cases, several definitions were required. Diarrhea was defined as illness in which at least three loose or liquid stools were passed in any 24-hour period. Visits for treatment of diarrhea were grouped into episodes if the date of onset of symptoms leading to a visit was within 7 days of the date of discharge from the previous visit. The onset of an episode was taken as the onset of symptoms leading to the initial visit of the episode. An episode of ETEC diarrhea denoted a diarrheal episode in which one or more lactose-fermenting colonies expressed either LT or ST during any component treatment visit. Because ETEC typically causes watery diarrhea, only episodes described as liquid or watery, without passage of visible blood, were included in the definition of ETEC diarrhea.

Severe ETEC diarrhea referred to an episode of ETEC diarrhea in which 1) the patient died during hospitalization; 2) the patient had significant dehydration, as manifested by at least two objective signs (feebly or absent radial pulse, poor skin turgor, sunken eyes, dry oral mucous membranes, depressed anterior fontanelle); or 3) the patient exhibited depressed mental status, as manifested by stupor or coma. All definitions were formulated before the study was undertaken, and all decisions about fulfillment of these criteria were made with use of a computerized algorithm executed without knowledge of breastfeeding status.

A child with severe ETEC diarrhea was potentially eligible to be a case if several additional criteria were met: 1) the onset of the episode occurred between January 1, 1985, and December 31, 1986; 2) the severe ETEC episode was the first to be detected for the child during the surveillance period; 3) the child was <36 months of age at the onset of the episode; 4) no fecal copathogens (V cholerae 01, Shigella, or rotavirus) were isolated during the episode; and 5) the child’s date of birth was after January 1, 1982, which made the child too young to be eligible for the vaccine trial. The fifth criterion was needed because one of the vaccines studied in the trial conferred protection against ETEC. A total of 632 episodes of ETEC diarrhea were detected in the target age group among Matlab residents during the study interval, of which 252 (39%) were episodes of severe ETEC diarrhea, for which solicitation of care could be presumed to be nondiscretionary, because limitation of the case group to episodes of severe ETEC diarrhea, for which solicitation of care could be presumed to be nondiscretionary, minimized the potential for selection biases related to differences in the use of the treatment centers by the Matlab population. To assemble cases, several definitions were required. Diarrhea was defined as illness in which at least three loose or liquid stools were passed in any 24-hour period. Visits for treatment of diarrhea were grouped into episodes if the date of onset of symptoms leading to a visit was within 7 days of the date of discharge from the previous visit. The onset of an episode was taken as the onset of symptoms leading to the initial visit of the episode. An episode of ETEC diarrhea denoted a diarrheal episode in which one or more lactose-fermenting colonies expressed either LT or ST during any component treatment visit. Because ETEC typically causes watery diarrhea, only episodes described as liquid or watery, without passage of visible blood, were included in the definition of ETEC diarrhea.

Selection of Controls

Control children were selected from three community surveys of the Matlab population, conducted in conjunction with the cholera vaccine trial in August and September 1985, November and December 1985, and March and April 1986. In each survey, 70 clusters of geographically contiguous families were randomly selected from census records of the Matlab Demographic Surveillance System. Home visits were made to collect sera and other relevant information, after acquisition of informed consent.18 Each of the resulting 210 clusters was unique and contained ~300 persons. In each cluster, 20 families having at least one child <5 years of age were randomly selected. We identified all 3759 children who were <36 months of age in these families at the times of the surveys; the 3 679 for whom dietary histories were obtained constituted the control group for this study.

Determination of Dietary Histories and Other Data

Dietary histories were collected in a uniform manner from mothers or care takers at the time of presentation for care for cases and at the time of the survey visits for controls. These data were collected by interviewers who were not aware of the study hypothesis. We defined a child as breastfed if breast milk constituted any portion of the child’s diet, as determined for the day before the onset of the episode for cases and for the day before the interview for controls. Exclusive breastfeeding denoted a diet in which no other solids or liquids were included. Other breastfed children were classified as partially breastfed. Height was measured to the nearest 0.1 cm during treatment visits for cases and during the surveys for controls, using identical supine length boards for children too young to stand and standing height sticks for older children. Height-for-age Z scores were calculated in relation to National Center for Health Statistics standards.20 Data about sociodemographic variables were collected during home visits for cases, shortly after the treatment visit, and during the surveys for controls.

Analyses

Comparisons of cases and controls for categorical variables were appraised statistically with the x2 test or the Fisher exact test for contrasts of binary variables in which the population was sparsely distributed. Contrasts of dimensional variables were evaluated with the Student’s t test or the Mann–Whitney U test when parametric assumptions were not fulfilled. To assess the strength of associations between breastfeeding and the risk of severe ETEC diarrhea, we calculated odds ratios. Because of the rarity of clinically severe ETEC diarrhea in Matlab children <36 months of age (1% risk over the study interval), odds ratios relating breastfeeding to severe ETEC diarrhea estimated the relative risk (RR) of severe ETEC diarrhea in breastfed versus nonbreastfed children.19 Estimations of 95% confidence intervals (CI) for these RR values were performed with test-based methods, or with exact methods when mandated by sparse data.22

To adjust the associations for the effects of potentially confounding variables, multiple logistic regression models were fitted, taking case–control status as the dependent variable and breastfeeding status, together with additional covariates, as independent variables. In these models, exponentiation of the coefficient for the variable for breastfeeding status enabled estimation of the adjusted RR (RR) relating breastfeeding to ETEC diarrhea, and 95% CI for these adjusted RR values were estimated with standard errors of the coefficients.22 Potential confounders considered for these models were demographic and nutritional features of the child, as well as demographic, socioeconomic, and hygienic characteristics of the household, that seemed plausibly related to the risk of treated diarrheal illnesses in young Bangladeshi children. Variables included as covariates in these models were those that were statistically related (P < .05) to case–control status in simple analyses, or, in lieu of statistically significant associations, those whose distributions in cases versus controls were judged to be quantitatively different. These included subject age, gender, and height-for-age Z score; family size; presence of a family tube well; and maternal age. Because of the relatively small number of non-breastfed cases and controls who were not breastfed during infancy, RR values were adjusted only for age in this age group to avoid overfitting the models to the data.23 However, inclusion of the additional potential confounding variables with age in these models yielded adjusted RR values that differed little from the age-adjusted RR values. All statistical tests were interpreted in a two-tailed manner.

RESULTS

Among ETEC isolates from the 168 cases, 42 (25%) expressed LT only, 56 (33%) expressed ST only, and 70 (42%) elaborated both toxins. A total of 68% of the cases presented with hyperacute symptoms (<2 days’ duration), and one (6%) died during hospitalization.

Comparability of Cases and Controls

Table 1 compares cases and controls for several sociodemographic and anthropometric features. In simple analyses, cases were significantly younger than controls and, corresponding to this younger age,...
As shown in Table 2, 155 (92%) of cases versus 3045 (83%) of controls were breastfed, leading to a crude RR of 2.48 (95% CI: 1.37, 4.61). However, after adjustment for potentially confounding variables, the RR declined to 0.86 (95% CI: 0.43, 1.74), indicating no significant association. Adjusted RR values were similar for comparisons of LT-only cases, ST-only cases, and LT+/ST+ cases with the entire control group (RRa = 0.85, 0.68, and 1.01, respectively).

The associations appeared to vary by age (Table 3). During infancy, both exclusive breastfeeding and partial breastfeeding were associated with an ~60% reduced risk of severe ETEC diarrhea, but neither of these associations reached statistical significance. However, in comparison with other feeding modes, exclusive breastfeeding was associated with a significant reduction of risk (RR = 0.51; P < .05; 95% CI: 0.28, 0.96). The protective association between exclusive breastfeeding (versus all other feeding modes) and the risk of severe ETEC diarrhea among infants was nearly identical for LT-only ETEC (RRa = 0.49; 95% CI: 0.15, 1.64), ST-only ETEC (RRa = 0.51; 95% CI: 0.17, 1.53), and LT+/ST+ ETEC (RRa = 0.54; 95% CI: 0.21, 1.38). Children who continued to be breastfed during the second and third years of life (all of whom were partially breastfed) did not experience any reduction in the risk of severe ETEC diarrhea (RRa = 0.98; 95% CI: 0.45, 2.12).

**Severe ETEC Diarrhea Versus Severe Cholera as Disease Outcomes**

Because *V. cholerae* 01 and ETEC can cause clinically indistinguishable watery diarrheas and because information obtained during surveillance was collected in an identical manner for all diarrheal cases blinded to diarrheal etiology, our surveillance provided a unique opportunity to compare the associations between breastfeeding and severe cholera versus severe ETEC diarrhea (Table 4). In these analyses, we selected cholera and ETEC cases from children <36 months of age who were detected during the 1985 to 1986 study interval; we used the definitions described earlier for diarrheal episodes, diarrheal severity, and breastfeeding status for both groups of cases; and we excluded cases with bloody diarrhea or with the following fecal copathogens: rotavirus and *Shigella* for both groups, *V. cholerae* 01 for the ETEC group, and ETEC for the *V. cholerae* 01 group.

A total of 126 eligible cases of severe cholera were identified, 63 of whom were breastfed (92%). Among severe cholera cases, breastfed cases were significantly younger than nonbreastfed cases (Z = –2.18, P = .03) and had significantly better height-for-age Z scores (Z = 1.33, P = .05). Cases also tended to have significantly younger mothers and to live in homes with a lower prevalence of tubewell water sources.

TABLE 2. Overall Association Between Breastfeeding and Severe ETEC Diarrhea

<table>
<thead>
<tr>
<th>Breastfeeding Status</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>Crude RR*</th>
<th>Adjusted RR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breastfed</td>
<td>155 (92%)</td>
<td>3045 (83%)</td>
<td>2.48† (1.37, 4.61)</td>
<td>0.86 (0.43, 1.74)‡</td>
</tr>
<tr>
<td>Nonbreastfed</td>
<td>13</td>
<td>634</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>168</td>
<td>3679</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Crude RR, and RR adjusted for subject age (coded directly in months), gender, and height-for-age Z score (coded directly); family size (coded as less than or equal to the control median vs larger than the control median) and presence of a family tubewell; and maternal age (coded as less than or equal to the control median vs larger than the control median).
† P < .01 (two-tailed) for comparison of cases and controls.
‡ 95% CI for the cited relative risk.
Our data suggest that breastfeeding of infants and children in this rural Bangladeshi population is associated with a rather meager overall reduction of the overall risk of severe ETEC diarrhea during the first 3 years of life. The small overall protective association (a nonsignificant 14% reduction of risk during the first 3 years of life) contrasts markedly with the ~70% reduction of the risk of severe cholera seen in these same Bangladeshi children. 10 The disparities in these levels of protection suggest that the immune and nonimmune protective properties of breastfeeding are far less effective against ETEC than against cholera in rural Bangladesh, despite the similar noninvasive pathogenesis of both infections and the known antigenic similarities of cholera toxin and ETEC LT. 24,25

Potential Limitations of the Study

Despite the large numbers of cases (168) and controls (3679) included in this study, the study was limited by the rather small number of nonbreasted children in both the case and the control groups. Nevertheless, the 95% CI for the overall RR, (0.43, 1.74; Table 2) excluded values (>57%) corresponding to high-grade protection. Moreover, despite the paucity of nonbreasted infants in the study, we found a statistically significant trend of increasing protective associations between breastfeeding and the risk of severe ETEC diarrhea with increasing exclusivity of breast milk in the diet, and a statistically significant association between exclusive breastfeeding per se (vs all other feeding modes) and the risk of severe ETEC diarrhea (Table 3). The last observation is important, because placement of partially breastfed infants in the group contrasted with exclusively breastfed infants made this contrast more conservative than the contrast between exclusive breastfeeding and no breastfeeding.

It might be argued that bias could have led to a depression of the protective relationship between breastfeeding and severe ETEC diarrhea in this study. Perhaps the strongest argument against such a bias is the fact that a strong protective relationship was found for breastfeeding and the risk of severe cholera in the same diarrheal surveillance system and community surveys used for the ETEC analysis (Table 4). This finding is persuasive, because in these two analyses, cases were clinically defined in the same way, the same control group was used, breastfeeding was defined identically, and the indistinguishable clinical features of severe ETEC diarrhea and severe cholera ensured that all dietary and other historical data were collected at the time of patient presentation without knowledge of diarrheal etiology.

Nevertheless, it could be reasoned that because the controls in our study were selected without reference to whether they experienced severe ETEC diarrhea during the study interval, the control group could have been contaminated by ETEC cases, leading to a false-negative relationship. However, our selection strategy for controls has been demonstrated to be appropriate for a case-control study designed to estimate the RR of an outcome in exposed versus nonexposed individuals. 21 Moreover, only 29 of the 3679 subjects in the control group were detected as having severe ETEC diarrhea during the study interval, and reanalysis of the data after exclusion of these subjects yielded virtually identical findings.

Relation to Previous Studies

Two previous studies suggested that titers of SIgA against LT in breast milk correlate inversely with the risk of diarrhea once an infant becomes infected by LT-producing ETEC. 26,27 One of these studies also found that the practice of breastfeeding was not associated with a reduced rate of colonization by ETEC per se, but was associated with a reduced rate of diarrhea among already colonized infants. 27

Our data extend these earlier findings in several ways. First, our study of 168 ETEC cases was considerably larger than either of the two earlier studies, which based their assessments on only 12 and 17 cases, respectively. Second, our findings indicate that a protective relationship was demonstrable during, but not after, infancy. Third, our data suggest that the breastfeeding–ETEC relationships applied con-
sistent to ETEC of all toxin phenotypes and to ETEC of life-threatening severity. Because the earlier studies limited their surveillance only to LT–ETEC and focused primarily on mildly symptomatic cases of ETEC, neither earlier study could address these features. Thus, although all three studies point to a biological effect of breastfeeding in reducing the risk of ETEC diarrhea during infancy, our study suggests that the overall magnitude of protection against severe ETEC diarrhea during the first 3 years of life is minimal—at least in rural Bangladesh.

Age-related Differences in Protection

Although the overall impact of breastfeeding on severe ETEC diarrhea during the first 3 years of life appeared modest, breastfeeding appeared to be associated with a lower risk of severe ETEC diarrhea during infancy. Because of the divergent relationships between breastfeeding and the risk of severe ETEC during and after infancy, it seems unlikely that the apparent protection during infancy was attributable to the rehydrating benefits of continued breastfeeding during diarrhea, because these benefits should also have been evident during the second and third years of life.

That the RR of severe ETEC diarrhea in exclusively breastfed infants versus infants on other diets was 0.51 suggests that introduction of supplemental foods and liquids was associated with a nearly twofold elevation of risk. Unfortunately, our dietary data were not detailed sufficiently to demarcate which foods or liquids seemed associated with the elevation of risk. Because breastfeeding was not associated with a lower risk of severe ETEC after infancy, the lower occurrence of severe ETEC in children 12 to 35 months of age (78 cases; Table 3) than in infants (90 cases) likely reflects age-related acquisition of natural immunity resulting from ETEC infection or perhaps some other developmental factor related to age, rather than an effect of breastfeeding.

Why should breastfeeding be associated with negligible protection against severe ETEC diarrhea after infancy, when both animal and human experiments indicate that ingestion of breast milk with high titers of antibodies to colonization factor antigens can protect against ETEC disease? One possibility is that the breast milk of Bangladeshi mothers of children in this age group contains insufficient quantities of secretory IgA antibodies against ETEC LT and colonization factor antigens—the factors regarded as primarily responsible for immune protection. Although titers of these antibodies in Bangladeshis have not been reported, one study from nearby Pakistan indicated that rather substantial titers of ETEC-specific antibodies are evident in mothers’ breast milk.

Regardless of the titers of such antibodies, it seems likely that a lack of protection in the second and third years of life could result from several age-related factors. For example, the transition from exclusive to partial breastfeeding may be accompanied by changes in the gut flora that may be more permissive for ETEC growth and may thereby overwhelm passive immune and nonimmune protection conferred by breast milk. An age-related decline in protection by breastfeeding might also be explained by increasingly diverse diets, leading to ingestion of greater and more frequent inocula of ETEC, and by progressive reductions in the volume of breast milk ingested.

Also working against the ability of the immune properties of breast milk to protect against ETEC is the diverse array of ETEC pathogens, expressing different combinations of toxins and colonization factors, that can coexist within a single endemic setting. Because protective immunity to these virulence factors appears to be largely homologous in specificity, mothers’ breast milk may contain insufficient titers to all prevalent ETEC virulence factors to provide complete coverage against all the ETEC pathogens to which children are commonly exposed. By contrast, the greater protective association of breastfeeding with cholera (Table 4) is consistent with the observation that protective antibacterial antibodies in breast milk are directed primarily against V cholerae 01 lipopolysaccharide, an antigen
that is nearly identical for all 01 serogroup cholera isolates.

Practical Implications

Our data suggest that promotion of breastfeeding cannot be relied on to have a major impact on the overall problem of life-threatening ETEC diarrhea in children residing in developing countries. For several reasons, however, this conclusion does not detract from the overall benefits of breastfeeding and the potential benefits of breastfeeding promotion in these settings. First, our analysis did not evaluate potential preventive effects of breastfeeding on mild ETEC illnesses, which, although not associated with life-threatening dehydration, may nevertheless be associated with growth-faltering.\(^1,4\) Second, although not proven, it is conceivable that the growth-faltering associated with ETEC may be more pronounced in infants and that the preventive impact of breastfeeding on ETEC infections in infants, shown in our data, could thereby be of great clinical importance. Finally, regardless of the magnitude of the preventive impact of breastfeeding on ETEC infections, the documented ability of breastfeeding to prevent other gastrointestinal infections in developing countries, such as cholera and shigellosis,\(^10,11\) as well as its overall salutary impact on infant and childhood mortality in these settings,\(^32,33\) provides strong support for breastfeeding promotion programs in these settings.

Contamination of weaning foods has been incriminated as a possible source of ETEC diarrhea in developing countries\(^34\) and educational interventions to improve hygiene in the preparation and storage of foods for infants and children may constitute a promising avenue for the control of ETEC.\(^35\) Another approach to preventing ETEC would be to vaccinate against this pathogen. However, because of the high occurrence of this disease in infancy, an ETEC vaccine would have to be effective when delivered during the routine course of infant immunizations. Progress has been made recently in the development of killed oral vaccines against ETEC,\(^36\) and field trials of the protective efficacy of one such vaccine candidate in Egyptian infants should begin in 1998.

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