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# *Shigellosis* among Breastfed Children: A Facility Based Observational Study in Rural Bangladesh

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## Abstract

**Objective:** Although breastfed children are less likely to suffer from infectious diarrhea, children under 2 years of age are often infected with *Shigella*. The study aimed to understand socio-demographic, clinical, and host characteristics of breastfed children under 2 years of age with shigellosis and compare these factors with breastfed children who presented with non-Shigella associated diarrhea in rural Mirzapur, Bangladesh.

**Methods:** From January 2010 to December 2012, a total of 3,409 children under 5 years with diarrhea were admitted to a tertiary level hospital in rural Bangladesh with diarrhea. A total of 2,278 (67%) of these children were aged 0-23 months and had reported history of breastfeeding and were enrolled in the study. Nine percent (n=205, 9%) of the enrolled children were infected with Shigella and were thus considered to be cases and the remaining children (n=2,073, 91%) were not infected with Shigella and formed the comparison group.

**Results:** Breastfed children with shigellosis were more likely to be underweight (<–2 weight-for-age z-score) [(31% vs. 18%; p<0.001)], stunted (<–2 height-for-age z-score) [(22% vs. 13%; p<0.001)] and wasted (<–2 weight-for-height z-score) [(22% vs. 13%; p<0.001)] compared to breastfed children without shigellosis. Rotavirus (14% vs. 29%, <0.001) was detected less commonly as a co-pathogen amongst children with shigellosis relative to their counterparts. In multivariate analysis, significant associations with shigellosis were observed with child age (12-23 months) [OR: 3.02 (95% CI-2.17-4.18)], blood in stool [OR: 6.44 (4.68-8.88)], fever [OR: 1.95 (1.41-2.68)], convulsion [OR: 2.80 (1.04-7.54)], stunting [OR: 1.53 (1.03-2.28)], and use of zinc at home [OR: 0.67 (0.46-0.98)] after controlling for other covariates.

**Conclusion:** Breastfed children with shigellosis were more likely to be malnourished than those who were also breastfed but not infected with Shigella among rural Bangladeshi children under 2 years of age.

**Keywords:** Breastfed; Diarrhea; Rural; *Shigella*; Children under 2 years

## Abbreviations:

DSS: Demographic Surveillance System; icddr,b: International Centre for Diarrheal Disease Research, Bangladesh; WHO: World Health Organization; ETEC: Enterotoxigenic *Escherichia coli*

## Introduction

Childhood shigellosis is a global public health problem [1]. Shigella, a Gram-negative bacterium, is a common cause of dysentery (bloody and/or mucoid diarrhea) and can have severe manifestations including hemolytic uremic syndrome, convulsions and death, particularly in young children [2,3]. Shigella causes dysentery by invading the colonic mucosa and multiplies within colonic epithelial cells, causing cell death and mucosal ulceration, inflammation and bleeding [1]. Approximately ca. 1.1 million deaths have been attributed to *Shigella* infections in developing countries, and 60% of the deaths occur among the young children annually [1]. The annual incidence rate of shigellosis in Bangladesh has been reported to be as high as 13.2 cases per 1000 children less than five years old [4], with particularly high rates in children under two years of age [5]. Shigellosis can have severe post infection nutritional consequences for children, including enteropathy associated with protein loss and slow growth and development among young children for up to one year post infection [6,7].

Breastfed children are known to be less likely to develop dysentery than non-breastfed children, and breastfeeding has been shown to reduce the severity of disease due to *Shigella* [8-10]. Breastmilk contains non-inflammatory secretory immunoglobulin A antibodies which protect against infection due to *Shigella*, Vibrio cholerae and Enterotoxigenic Escherichia coli (ETEC) [11]. In Bangladesh, about one third of children are breastfed up to three years of age [9]. Despite the evidence of protection however, breastfed children under two years of age continue to present with dysentery at both outpatient and inpatient clinics in Bangladesh. A recent literature review identified those children less than two years in rural areas of Bangladesh to be

especially vulnerable to infection with *Shigella* [5,12,13]. Therefore, we have designed a study to investigate risk factors for shigellosis among rural Bangladeshi children who are breastfed. Specifically we aimed to examine socio-demographic, clinical and nutritional characteristics and distribution of co-pathogens of breastfed children presenting to hospital diagnosed with *Shigella* and compared these factors against breastfed children diagnosed with alternate diarrheal etiologies.

## Materials and Methods

### Study site

The study was conducted at the Kumudini Women's Medical College and Hospital, located in a rural community of Mirzapur sub-district, Bangladesh, approximately 40 miles northwest of the capital city Dhaka. The International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) has established a Demographic Surveillance System (DSS) in Mirzapur and collects longitudinal information on vital events, such as birth, death, marriage, and migration. The community has a population of over 263,000 and 3% percent of the DSS population is comprised of children under two years of age [12].

### Study design and participants

From 2010 to 2012, a total of 3,409 children under five years of age with diarrhea presented to the hospital. Information on current breastfeeding practices was documented, including whether the mother breastfed during an illness in the child. For inclusion into the case control analysis, children needed to be under 24 months of age and known to be breastfed. A total of 2,278 (66.8%) were enrolled in this study from the original 3,409 presenting to hospital. We categorized children with diarrhea into two groups according to presence or absence of *Shigella* in stool specimen. Of them, 9% (n=205) were positive for *Shigella* whilst 91% (n=2,073) were not. Information on socio-economic and demographic characteristics, feeding practices, and use of drug and fluid therapy at home were collected by trained research assistants by administering a field-tested questionnaire. Additionally, information on clinical characteristics, anthropometrics, and treatment received at facilities and outcome of patients were also recorded.

### Specimen collection and laboratory procedures

A single, fresh, whole stool specimen (at least 3 ml or grams, ideally 10 ml or grams) was collected from patients at enrollment. Additionally, a fecal swab was placed in Cary-Blair medium in a plastic screw top test tube and each specimen was packed and labeled with the subject's identification number, date and time of collection. Using a styrofoam container with cold packs, the specimen was transported to the central laboratory of icddr,b in Dhaka for isolation and identification of rotavirus-specific VP6 antigen was detected in the stool specimens using a solid-phase sandwich-type enzyme immunoassay (EIA) modeled after the ProSpecT commercial kit assay (Oxoid Ltd, Basingstoke Hants, United Kingdom). Positive and negative controls were included in every test run. Quality control of the EIA test was routinely performed using rotavirus-positive samples with known optical density (OD) values [14,15], Enterotoxigenic *Escherichia coli* (ETEC) in stool samples were plated onto MacConkey agar, and the plates were incubated at 37°C for 18 hours. Six lactose-fermenting individual colonies morphologically resembling *E. coli* were tested [16,17], Vibrio cholera, the stool samples were plated on

taurocholate-tellurite-gelatin agar [18] and gelatin agar (Difco, Detroit, MI); after overnight incubation of plates, serological confirmation of suspected *Vibrio* colonies was carried out by slide agglutination [19,20], and *Shigella* spp.was isolated and identified according to standard microbiological and biochemical methods. *Shigella* spp. was also confirmed and serotyped using a commercially available antisera kit (Denka Seiken, Tokyo, Japan). In cases of non-typeable *S. flexneri*, serotyping was also performed by using monoclonal antibody reagents specific for all type and group factor antigens [21,22] following standard methods.

## Ethical Statement

The Research Review Committee (RRC) and the Ethical Review Committee (ERC) of International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) approved the present study under protocol entitled "Disease burden and etiology of diarrhoea patients visiting Kumudini Hospital, Mirzapur". Informed written consent has been taken from the caregiver of each study children prior to enrollment. All the information you give us will be kept in a locked cabinet. Study staff, authorized persons from the sponsor of the study, ethical review committees, the Ministry of Health of Bangladesh, and regulatory agencies may also see your child's personal study data or other medical records. This written consent was documented by keeping a check mark in the questionnaire which was again shown to the parents. Parents or guardians were assured about the non-disclosure of information collected from them, and were also informed about the use of data for analysis and using the results for improving patient care activities as well as publication without disclosing the name or identity of their children. ERC was satisfied with the written participation, maintenance of all the rights to participate in the study or may withdraw your child from this study at any time and you will not deprive from our usual treatment. Confidential handling of personal information by the study physicians and has approved this consent procedure.

## Definitions

**Diarrhea and dysentery:** Diarrhea was defined as three or more loose, liquid, or watery stool while dysentery was defined as at least one loose stool containing blood in a 24-hour period [12].

**Malnutrition:** According to World Health Organization (WHO) growth standards, children were considered malnourished if they were underweight (<–2 weight-for-age z-score); stunted (<–2 height-for-age z-score); wasted (<–2 weight-for-height z-score) [23]. Breastfeeding was defined as continuing breastfeeding at point of enrollment.

## Data analysis

Statistical analyses and data entry were performed using Statistical Package for Social Science (SPSS, Chicago, IL version 15.5) and Epi Info (Version 6.0, USD, Stone Mountain, GA). For categorical variables, differences in the proportion were compared by Chi-square test. A probability of less than 0.05 was considered to be statistically significant. A backward stepwise logistic regression analysis was performed with the probability of exclusion at p=0.10 to identify the factors significantly associated with dependent variable (breastfed Shigella vs. breastfed non-*Shigella*). The covariates used in the model were child age, sex, maternal illiteracy, occupation of father, total monthly income, household sanitation, use of soap after defecation, duration of diarrhea, presence of blood in stool, vomiting, abdominal

pain, rectal straining, cough, fever, history of convulsion, use of oral rehydration solution (ORS), zinc and antimicrobials at home, seeking care prior to hospital visit, stunting, and other etiologies of diarrhea (Vibrio cholerae, rotavirus and ETEC).

## Results

Of the 205 enrolled breastfed children with shigellosis, 59% were male compared to 61% of the 2,073 non-shigellosis breastfed children. A significantly higher proportion of breastfed children with Shigella were aged 12-23 months (68%) compared to breastfed children without Shigella (39%). Use of ORS (76% vs. 80%) and reported antimicrobial treatment (38% vs. 40%) at home or community level was similar in both the groups. A lower proportion of children with shigellosis used zinc (22%) before coming to the hospital (34% in non-shigellosis group), whilst immunization coverage and vitamin A supplementation and there were no significant differences in sex, illiteracy of mother, father stayed outside home, monthly income, household sanitation, use of soap after defecation, no drinking water, use of oral rehydration solution (ORS), antimicrobials at home and seeking care prior to hospital visit among the groups (Table 1). Breastfed children with shigellosis were more likely to be underweight, wasted and stunted compared to their counterparts (Table 1).

| Variable | Breastfed with nShigella n=205 (%) | Breastfed non-Shigella n=2073 (%) | OR (95% CI) | P value |
|---|---|---|---|---|
| Male sex | 121 (59) | 1254 (61) | 1.06 (0.79, 1.44) | 0.737 |
| Illiteracy of mother | 19 (9) | 210 (10) | 0.91 (0.54, 1.52) | 0.787 |
| Age stratum | | | | <0.001 |
| 0-11 month | 65 (32) | 1268 (61) | 3.39 (2.47, 4.67) | |
| 12-23 month | 140 (68) | 805 (39) | | |
| Father stayed outside home | 34 (17) | 436 (21) | 0.75 (0.50, 1.11) | 0.158 |
| Monthly income <100 US$ | 101 (49) | 1125 (54) | 0.82 (0.61, 1.10) | 0.194 |
| Use of sanitary toilet | 71 (35) | 690 (33) | 0.94 (0.69, 1.29) | 0.754 |
| Used soap after defecation | 150 (73) | 1579 (76) | 0.92 (0.65, 1.32) | 0.718 |
| No drinking water treatment | 192 (94) | 1941(94) | 1.00 (0.54, 1.90) | 0.892 |
| Prior health care sought[1] | 168 (82) | 1724 (83) | 1.09 (0.74, 1.60) | 0.73 |
| Prior ORS use[1] | 155 (76) | 1653 (80) | 1.27 (0.89, 1.80) | 0.718 |
| Prior zinc use[1] | 44 (22) | 695 (34) | 1.85 (1.29, 2.65) | <0.001 |
| Prior antimicrobial use[1] | 78 (38) | 836 (40) | 0.91 (0.67, 1.23) | 0.575 |
| Travel >5 miles | 97 (53) | 866 (42) | 1.25 (0.93, 1.69) | 0.144 |
| Measles vaccination[2] | 133 (95) | 763 (95) | 1.05 (0.44, 2.61) | 0.92 |
| Vitamin-A capsule[2] | 122 (87) | 722 (90) | 0.78 (0.44, 1.40) | 0.452 |
| Nutritional status[3] | | | | |
| Underweight | 64 (31) | 369 (18) | 2.10 (1.51, 2.91) | <0.001 |
| Stunted | 44 (22) | 264 (13) | 1.87 (1.29, 2.72) | <0.001 |
| Wasted | 44 (22) | 267 (13) | 1.85 (1.27, 2.68) | <0.001 |

**Table 1:** Socio-demographic characteristics of breastfed children aged 0-23 months with and without *Shigella* infection. [1]Prior to hospital visit; [2]For children 12-23 months of age; [3]Underweight (<–2 weight-for-age z-score), stunted (<–2 height-for-age z-score), wasted (<–2 weight-for-height z-score).

Differences in clinical presentation between breastfed children with and without Shigella were apparent. Children with shigellosis presented more often with abdominal pain, rectal straining, blood in stool, and fever compared to non-shigellosis children. Four percent of Shigella infected children had convulsions, which was significantly more common compared to those without Shigella. Vomiting was less likely to be reported by children with Shigella infection. There were no differences in cough, stool frequency and duration of diarrhea among the groups (Table 2).

| Variable | Breastfed with Shigella n=205 (%) | Breastfed non-Shigella n=2073 (%) | OR; (95% CI) | p value |
|---|---|---|---|---|
| Blood in stool | 135 (66) | 439 (21) | 5.85 (4.27, 8.02) | <0.001 |
| Vomiting | 88 (43) | 1158 (56) | 0.59 (0.44, 0.80) | <0.001 |
| Abdominal pain | 159 (78) | 1347 (65) | 1.86 (1.31, 2.66) | <0.001 |
| Rectal straining | 145 (71) | 849 (41) | 3.48 (2.52, 4.83) | <0.001 |
| Cough | 83 (41) | 934 (45) | 0.83 (0.61, 1.12) | 0.237 |
| Fever | 128 (62) | 884 (43) | 2.24 (1.65, 3.04) | <0.001 |
| Convulsion | 9 (4) | 13 (1) | 7.28 (2.83, 8.44) | <0.001 |
| Excessive stool frequency[1] | 77 (38) | 756 (37) | 1.05 (0.77, 1.42) | 0.815 |
| Duration of diarrhea | | | | |
| 0-72 hrs | 146 (71) | 1504 (73) | 1.07 (0.77, 1.48) | 0.744 |
| >72 hrs | 59 (29) | 569 (27) | | |

**Table 2:** Clinical characteristics with nutritional status of breastfed children aged 0-23 months with and without *Shigella* infection. [1]>10 stools/24 hours.

Among those with shigellosis, 52% were classified as Shigella flexneri followed by Shigella sonnei (27%), Shigella boydii (17%), and Shigella dysenteriae (4%). A significantly lower proportion of children with Shigella were co-infected with rotavirus (14% vs. 29%) compared co-infections among those without Shigella infection (Table 3). Proportion of ETEC and Vibrio cholerae was similar (Tables 3 and 4).

| Pathogen | Breastfed with Shigella n=205 (%) | Breastfed non-Shigella n=2073 (%) | OR; (95% CI) | p value |
|---|---|---|---|---|
| Rotavirus | 29 (14) | 596 (29) | 0.41 (0.27, 0.62) | <0.001 |
| ETEC | 7 (3) | 73 (4) | 0.97 (0.40, 0.22) | 0.904 |
| V. cholerae | 7 (3) | 33 (2) | 2.19 (0.87, 5.24) | 0.084 |

**Table 3:** Distribution of pathogens among breastfed children aged 0-23 months with and without *Shigella* infection.

In multivariate analysis, significant associations were observed between Shigella infection and child age, blood in stool, fever, convulsion, stunting, and use of zinc at home after controlling for other variables such as sex, maternal illiteracy, occupation of father, total monthly income, household sanitation, use of soap after defecation, duration of diarrhea, vomiting, abdominal pain, rectal straining, cough, use of oral rehydration solution (ORS), and antimicrobials at home, seeking care prior to hospital visit, and other etiologies of diarrhea (Vibrio cholerae, rotavirus and ETEC).

| Indicator | Adjusted OR (95% CI) | p value |
|---|---|---|
| Age 12-23 months | 3.02 (2.17, 4.18) | <0.001 |
| Blood in stool | 6.44 (4.68, 8.88) | <0.001 |
| Fever | 1.95 (1.41, 2.68) | <0.001 |
| Convulsion | 2.80 (1.04, 7.54) | 0.041 |
| Prior zinc use[1] | 0.67 (0.46, 0.98) | 0.039 |
| Stunting[2] | 1.53 (1.03, 2.28) | 0.033 |

**Table 4:** Factors associated with *Shigella* infection among breastfed children aged 0-23 months in multivariate analysis. [1]Before attending hospital; [2]<-2 height-for-age Z score.

## Discussion

The most interesting and important observation of the present study was that, despite breastfeeding, children under two years of age infected with *Shigella* were more malnourished compared to breastfed non-Shigella children. Children with *Shigella* were perhaps more susceptible to infection because they were malnourished or malnutrition could be consequence of Shigella infection. Thus, we could speculate that nutritional interventions are equally important along with promotion of breastfeeding to reduce the burden of diarrhea in children less than two years old [24,25].

Shigellosis among breastfed children could be explained by several ways. A primary reason could involve a lack of immunity against *Shigella* infection. Human breast milk protects infants against *Shigella* infection when it contains high concentration of secretory IgA against virulence plasmid-associated antigens [26]. However, breast milk among malnourished children may not give optimal protection [27,28] or exclusive breastfeeding may be unlikely to be maintained beyond six months of age. It has been well documented that breastmilk confers protection against diarrhea up to for the first 6 months of life but weaning during this period puts the infants at risk for exposure to food borne enteric pathogens. This is due to the introduction of potentially contaminated food or drink and low inoculums size because of immature immune priming [29]. Other than that, the pathogen itself may change its virulence characteristics that may cause changes of the host immunity [30]. Bacterial pathogens such as *Shigella*, are able to efficiently colonize the intestinal epithelium by utilizing highly evolved mechanisms to counteract host innate defense mechanisms [31]. Previous studies have reported that enteric bacterial pathogens possess distinctive mechanisms to attenuate host inflammatory responses, which is a prerequisite for promoting intracellular and extracellular bacterial survival [32,33]

Moreover, diarrhea among breastfed children is sometimes observed due to lactose intolerance and children might pass green, watery, or loose stool which is usually misperceived by the mothers as diarrhea stool [34,35]. This reiterates the importance of extensive counseling and proper guidance about food hygiene and safety before and during the introduction of complementary foods.

Shigellosis is usually more common in older children [36]. Our study confirmed this observation with higher number of cases with Shigella infection among children in the second year of life. There is no readily explanation in this regards, but teething and introduction of contaminated substances for gingival etching could be a strong cause of such high rate infection with shigella in this age group. We could speculate another way that older age group is vulnerable to infected with shigella may be caught by drinking water contaminated with infected faeces, eating food prepared using contaminated water or by close contact with someone who has the infection or environmental expose. Furthermore, children in this study presented with features which were similar to those reported in other literature [37]. Namely, passage of small volume, non-watery loose and bloody-mucoid stools, abdominal cramps, rectal straining, and fever [38]. Moreover, children in this study with shigellosis were at 3 times higher risk for development of convulsion which has also been supported by previous studies [3,39]. However; no differences in cough, stool frequency and duration of diarrhea among the groups.

The study identified that children infected with *Shigella* were less likely to be co-infected with rotavirus This interesting observation is difficult to account except nutritional status differentials. Low detection of rotavirus as co-pathogen may be due to presence of one enteric pathogen i.e. *Shigella* which is invasive in nature [33]. Indeed, shigellosis causes growth retardation, worsening of malnutrition, and hypoproteinemia [40,41] which might have reduced the number of gut receptors, thereby causing less frequent cellular attachment at gut mucosal level of rotavirus [42]. However, it may also be that differing seasonality and epidemiology of the two pathogens accounts for the difference in co-pathogen isolation rate. Isolation rates for ETEC and Vibrio cholerae were similar in both the study groups.

There were no significant differences in sex, illiteracy of mother, father stayed outside home, monthly income, household sanitation, use of soap after defecation, no drinking water, use of oral rehydration solution (ORS), antimicrobials at home and seeking care prior to hospital visit among the groups. Children with shigellosis were found to have used zinc at home less often. Zinc is widely recommended for limiting stool frequency as well as duration of the episode [43]. It is recommended for use at community level upon onset of diarrhea because of its potential immune boosting properties [4,43,44]. in currently introduced guideline for the control of shigellosis to including zinc as an adjunct therapy and increased immune response with elevated *Shigella* antigen specific IgG responses and lymphocyte proliferation responses and higher serum shigellacidal antibody titer, play role in growth limiting agent against *Shigella* isolates [43,45]. Less frequent use might be explained by several reasons, including the fact that mothers may consider dysentery as a severe disease and seek health care immediately to the health facility, the unavailability of the zinc at community level or lack of knowledge about zinc usage for bloody diarrhea.

This study has several limitations. Firstly, a lack of information regarding extent and duration of exclusive breastfeeding and history of weaning practices at the household level may have biased our findings. Information collected based on mothers' perception or reporting without observation at household level might further add to our limitations. Data collected by questionnaire to the mother in observational conditions is truly a strong limitation in many medical studies. Questionnaire to the source of study are being used nowadays repeatedly in performing different analytical studies which could cause in many cases limitation to such studies. However, unbiased enrollment, irrespective of sex, nutritional status, disease severity, and socio-economic background along with a large dataset with quality laboratory performance were the strengths of the current study.

## Conclusion

Breastfed children with *Shigella* infection presenting to hospital were more malnourished than breastfed children without *Shigella* infection. Such results indicate that through breastfeeding a child may not be fully protective against shigellosis an infection which can have severe adverse nutritional outcomes. Breastfeeding is very important in many aspects for protecting and achieving better health to the baby. That does not allow full protection against many microbial and viral born diseases. This requires that other precautionary measures must be implemented such as mother hand washing and disinfect ion of the aria where the baby could play-in. Thus, the present study draws the attention of the policy makers with emphasis to conduct further large scale analogous studies in different facilities. This may help to explore knowledge gap for implementation of strategic plan to scale up exclusive breastfeeding practices for 6 months and continuation of the breast feeding practices with an emphasis on complementary feeding as well as a step to add value to effort being made to improve the

current management protocol for malnourished children including follow-up at the community level to reduce the burden of diarrheal illness due to Shigella infection.

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