The impact of household hygiene on the risk of bacterial diarrhea among Egyptian children in rural areas, 2004–2007

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Abstract

Introduction: The present study, conducted between January 2004 and April 2007, explored the impact of household hygiene on the risk of bacterial diarrhea, using data from a prospective birth cohort of 348 infants in five villages in the Nile Delta in Egypt.

Methodology: Neonates were enrolled at birth and followed up until 24 months of age. Children were visited twice a week to survey them for acute diarrhea. A detailed observational household hygiene survey was completed in-house every six months. Adjusted relative risk (aRR) of developing bacterial diarrhea was calculated for exposure to different hygiene variables and examined for specific bacterial pathogens.

Results: Exclusive breastfeeding reduced the risk of bacterial diarrhea by 70%, while bacterial diarrhea cases were 2.6 times higher in the warm season. Risk of Campylobacter diarrhea increased with the absence of barriers to keep birds and animals out of the eating area, the presence of garbage containers and a bathing facility within the compound, and the presence of feces on the floor of the bathing facility. Use of municipal water for drinking and cooking was associated with a lower risk of Campylobacter diarrhea. Risk of enterotoxigenic Escherichia coli diarrhea increased with uncovered garbage containers and the presence of liquid materials in the garbage containers, but decreased with the use of tap water in the washing facility.

Conclusion: The results highlight some potential targets for interventions, such as expanding municipal water supply to all houses and comprehensive mass-media awareness programs to change hygiene-promoting behaviors and practices.

Key words: Egypt; household hygiene; bacterial diarrhea.


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Introduction

Diarrheal diseases are a leading cause of child morbidity worldwide. They were the third leading cause of death among populations of low-income countries in 2004, and the second leading cause of death among children under five years of age, responsible for 6.9% of the overall deaths and for the deaths of 1.5 million children every year, of which 80% are estimated to occur among those under two years of age [1-3]. Infectious diarrhea is caused by a variety of bacterial, viral, and parasitic organisms. Diarrheagenic Escherichia coli, Campylobacter, Shigella, and Salmonella are the most common causes of bacterial diarrhea, and are known to spread through contaminated food or drinking water, as well as due to poor hygienic conditions [4,5]. Water contaminated with human feces from sewage, septic tanks, and latrines is of particular concern. Animal feces also contain microorganisms that can cause diarrhea [3]. Diarrheal diseases can also spread from person to person, aggravated by poor personal hygiene. Food is another major cause of diarrhea when it is prepared or stored in unhygienic conditions [3,6].

Worldwide, around one billion people lack access to safe water and 2.5 billion have no access to basic sanitation [4,5]. Additionally, maternal hygiene behaviors relating to childcare practices, such as feeding, hand washing, and cleaning, may have an impact on the risk of diarrhea in children [6,7]. Contaminated drinking water or food, suboptimal water sanitation, and poor domestic and personal hygiene are often linked to childhood diarrheal illness in developing countries [4,9]. While improvements in access to clean water and sanitation are of critical importance as defined by the Millennium Development Goals [10], progress has been challenged; therefore, other potential avenues of disease mitigation are important to understand, and
might serve as methods of intervention. Therefore, in this study, we attempted to explore the association of household and personal hygiene factors with the risk of bacterial diarrheal illness among a pediatric cohort. Four bacterial pathogens were identified for this analysis: enterotoxigenic Escherichia coli (ETEC), Campylobacter, Shigella, and Salmonella.

Methodology

Study population and enrollment
A prospective birth cohort study of 348 infants was conducted between January 2004 and April 2007 in five villages located in Abu Homos, a rural district in the Nile Delta in northern Egypt. A census was performed for 1,916 households with a total population of 15,675 inhabitants. Neonates were enrolled at birth and followed up to 24 months of age.

Diarrhea surveillance
Children were visited at home twice a week to survey for acute diarrhea (weekly surveillance). At each visit, a social worker asked the caretaker about diarrheal symptoms in the enrolled child that had occurred since the previous visit. The child's caretaker was interviewed about the severity of the illness, about any other illnesses, and about feeding patterns. If any loose or liquid stools were reported, a fecal specimen and two rectal swabs were collected.

During follow-up, all children were vaccinated with polio and DPT vaccine when they were two, four, and six months of age according to the national vaccination program for children [11]. All children had anthropometric measures every two months when they visited the clinic.

Hygiene survey
A detailed observational survey of the household’s hygiene conditions was completed at the child’s home, by a trained social worker, approximately every six months during the two-year follow-up period. The hygiene questionnaire consisted of ten sections, including 74 variables and specific observed hygiene behaviors. These included the hygiene characteristics and conditions of areas for sleeping, eating, cooking, washing and bathing, and bathroom facilities. The presence and utilization of garbage containers, the presence and preservation conditions of previously prepared food, water sources and containers utilized, as well as the presence of domestic animals and flies in the house were also determined through the questionnaire.

Specimen collection and transportation
Stool or rectal swabs were collected at each visit whenever a symptomatic illness was reported. Two rectal swabs were collected; the first swab was placed in Cary-Blair (CB) transport media and the second swab placed in a tube of buffered glycerol saline (BGS). In addition, attempts were made to collect a stool sample to be used for viral and parasitic analyses. All specimens were transported immediately on ice packs to the Abu Homos field laboratory for processing. Rectal swabs were refrigerated at 2°C to 8°C, while stool samples were aliquoted and stored at -20°C. All laboratory specimens were sent to the United States Naval Medical Research Unit No. 3 (NAMRU-3) in Cairo within three days of collection via a consistent cold chain. Frozen samples were stored at -70°C until testing.

Laboratory evaluation and procedures
The CB swabs were plated on MacConkey, Salmonella-Shigella (SS), Campylobacter blood, and thiosulfate citrate bile salts sucrose (TCBS) agar plates, while BGS swabs were plated only on MacConkey and SS agar plates. All primary agar plates were incubated overnight (except for Campylobacter blood agar plates, for which the incubation period was 48 hours) and were checked for growth the following day. Conventional microbiological procedures were used to isolate and identify Shigella, Salmonella, and Campylobacter spp. In addition, five lactose-fermenting colonies with E. coli-like morphology picked from the primary MacConkey agar plate were stored in trypticase soy broth with 15% glycerol until tested for ETEC heat-labile (LT) [12] and heat-stable (ST) enterotoxins using GM1-enzyme-linked immunosorbent assays (GM1-ELISAs) [12,13].

Definitions
A diarrheal day was defined as the occurrence of at least three non-formed stools (or at least one if bloody) in a 24-hour period. In addition, if the child was breastfed and the stool was not bloody, the mother had to report an increase in frequency or a reduction in consistency of the stools, compared with what she considered to be normal. A diarrheal episode was defined as beginning on the first day of loose or liquid stools after at least three consecutive non-diarrheal days and ending when followed by at least three consecutive non-diarrheal days.

“Crowding” assessed the number of persons per sleeping room in a household, and was classified into
two categories: moderate-high (number of persons per sleeping room was \( \geq 3.5 \)) versus low (number of persons per sleeping room \( < 3.5 \)) [14].

“Season” was classified as the warm season, from 1 May to 31 October; otherwise, the season was classified as cold.

Regarding maternal education, all mothers able to read and write (regardless of their formal educational levels) were classified as literate; otherwise, mothers were classified as illiterate. The classified literacy level was considered as sufficient to understand the simple messages and to absorb the future interventions to promote community and personal hygiene.

Regarding socioeconomic status (SES) of families, direct questions about a family’s income were not asked, as inaccurate replies are often obtained. Instead, an indirect approach was used to determine the socioeconomic status of the family. The approach depended on the number of luxury items, transportation means, land and farm ownership, animal and bird possession, and any other elements generating income for the family. Each item was scored. All scores were summed and classified into three categories: low, moderate, and high SES.

Stunting is defined as having a height (or length) for age of more than two standard deviations below the median of the National Center for Health Statistics/World Health Organization (WHO) growth reference.

Analytical methods

Four household hygiene surveys were conducted during the study period. Data from household hygiene surveys were linked to data from twice-weekly surveillance. Each diarrhea episode was linked to the corresponding bacteriology result and to data from the previous hygiene survey. Only diarrhea cases from sole bacterial pathogens were selected for analysis (i.e., diarrhea infections due to mixed pathogens or other causes were excluded) to avoid the differential impact of some hygiene factors on co-pathogen diarrhea episodes.

Statistical analysis

Two steps of statistical analysis were applied. In the first step, a trial was made to explore the impact of common socioeconomic, demographic, and environmental factors, as quoted in previous literature and reports [15-18] on the risk of diarrhea developing among children. These factors included the child’s age, child’s sex, breastfeeding status of the child, crowding in the house, season of infection, maternal education, and socioeconomic status of the family. Only socioeconomic, demographic, and environmental factors significantly associated with the risk of each bacterial-diarrhea pathogen were selected as adjustment factors in the second step of the analysis.

In the second step, crude relative risks (cRRs) were calculated for the risk of diarrhea developing with specific bacterial pathogens, computed as a ratio of the incidence rates in the presence and absence of each of the 74 hygiene factors under study. The incidence rate was determined by dividing the number of diarrhea episodes with a sole bacterial pathogen by total person-years at risk. Each household hygiene factor that had a significant association with the risk of bacterial diarrhea pathogens (measured by cRR at a statistical significance level of \( p < 0.10 \)) was adjusted by the significant confounding variables that appeared from the first analysis step, as well as in multiple observations of individual subjects, leading to adjusted relative risks (aRRs) [18]. Only hygiene variables that had significant associations with bacterial diarrhea (either by cRR or aRR with a statistical significance level of \( p < 0.05 \)) were tabulated. The analysis included only children who completed at least one household hygiene survey. For the purpose of analysis, some factors of the hygiene survey could be considered time-varying covariates. Therefore, the exposure period associated with a given survey was considered to be associated with the pathogen-specific illness risk in the period subsequent to the survey. Poisson regression models, with the use of generalized estimation equations (GEE) to adjust for the repeated individual subjects, were fitted, using SAS Proc GENMOD with GEE equation, SAS version 9.1 (SAS Institute Inc., Cary, USA).

Protection of human subjects

Before enrollment, written informed consent was obtained from the mother, father, or guardian of the eligible child. The study protocol IRB No. 145 (DOD No. NAMRU3.2003.0011) was approved by the NAMRU-3 Institutional Review Board in compliance with all applicable federal regulations governing the protection of human subjects. The protocol was also approved by the Egyptian Ministry of Health and Population.

Results

Bacterial diarrhea incidence rates

During the study period, January 2004 to May 2007, 348 children were enrolled in the study for a twice-weekly surveillance. The overall number of
diarrhea episodes identified was 4,001 episodes, with an overall incidence rate of 7.8 episodes per child-year. One-fourth of all diarrhea cases were identified as bacterial diarrhea (999 episodes) with an incidence rate of 1.95 bacterial diarrhea episodes per child-year. Of all infections, 924 were sole bacterial pathogen infections, while the most frequent multi-pathogen combinations found included ETEC/Campylobacter. The overall incidence rate of bacterial diarrhea per child-year was distributed as 1.24 for sole ETEC diarrhea (632 episodes during the study period), 0.47 for sole Campylobacter diarrhea (240 episodes), 0.08 for sole Shigella diarrhea (42 episodes), 0.02 for sole Salmonella diarrhea (10 episodes), and 0.14 for mixed bacterial diarrhea (75 episodes of any combination of the above-mentioned pathogens). Because of small numbers and instability of effect estimates, hygiene-associated risks of Shigella and Salmonella illnesses were excluded from further analysis.

At least one anthropometric measure was performed for 311 out 348 children, and 173 children had all the anthropometric surveys. Thirty-six out of 311 (11.6%) were stunted by the end of the follow-up period. 

Coverage of household hygiene information
Household hygiene information was obtained from 90% of the enrolled children (n = 315), who completed at least one of the four hygiene surveys conducted during the study period. Eighty-five percent of bacterial diarrhea cases were linked with the available corresponding hygiene surveys (852/999 episodes). Eighty-five percent of sole ETEC diarrhea and 84% of sole Campylobacter diarrhea (540 and 201 episodes, respectively) were linked to data from the corresponding hygiene surveys.

Confounding variables
All potential socioeconomic, demographic, and environmental confounders were selected and examined for their association with the risk of diarrhea due to different bacterial pathogens (Table 1). The child’s age, gender, breastfeeding status, crowding at the child’s home, season of diarrhea, and maternal education were significantly associated (p < 0.05) with the risk of diarrhea. Breastfeeding and seasonality of disease were strongly associated (p < 0.001) with the risk of ETEC diarrhea. Exclusive breastfeeding was found to reduce the risk of ETEC diarrhea (aRR 0.5, p < 0.001). Risk for ETEC diarrhea was significantly increased during the warmer season (aRR 2.9, p < 0.001). Male children were at higher risk of ETEC diarrhea than were female children (aRR 1.2, p < 0.05). Children of educated mothers were found to be at less risk of developing ETEC diarrhea (aRR 0.8, p < 0.05) (Table 1). Children in their first year of age were at a higher risk for developing Campylobacter diarrhea than were older children (aRR 3.9, p < 0.001), while exclusive breastfeeding of the child was significantly associated with a lower risk of Campylobacter diarrhea (aRR 0.2, p < 0.001). Crowding and warm season were also found to have a significant association with an increased risk of Campylobacter diarrhea (aRR 1.5, p < 0.01 and aRR 1.6, p < 0.01, respectively) (Table 1-S).

No significant impact of the socioeconomic status of the families was observed. All demographic confounding variables that appeared to be significantly associated (p < 0.05) with the risk of each bacterial pathogen were used as adjustment variables in the prediction model of the impact of hygiene variables on the risk of diarrhea. 

Hygiene variables
Among the 74 variables and hygiene behavior observed, 10 variables were significantly associated with the risk for bacterial diarrhea (Table 2). These variables were classified into four categories.

Hygiene behaviors and practices of eating rooms
Campylobacter diarrhea was the only bacterial pathogen influenced by the hygiene conditions of eating rooms. Among six variables related to hygiene behaviors and conditions of eating area, the lack of barriers to keep birds and animals out of the area was found to be significantly associated with a high risk for Campylobacter diarrhea (aRR 1.47, p < 0.05). None of these sets of hygiene behaviors and practices were found to be significantly associated with the risk of ETEC diarrhea, all bacterial diarrhea, or all diarrhea cases.

Hygiene behaviors and practices related to garbage containers
Bacterial diarrhea was found to be affected by two out of six variables related to the hygienic state of garbage containers. Uncovered garbage containers and liquid materials in the garbage containers were found to be significantly associated with an increased risk for bacterial diarrhea (aRR 1.4, p < 0.01 and aRR 1.6, p < 0.001, respectively) and more specifically, with ETEC diarrhea (aRR 1.6, p < 0.05 and aRR 1.4, p < 0.05, respectively).
Table 1. Association between socioeconomic, demographic, and environmental variables and risk of bacterial diarrhea among children under two years of age

<table>
<thead>
<tr>
<th>Variables</th>
<th>Age</th>
<th>Sex</th>
<th>Breastfeeding</th>
<th>Crowding **</th>
<th>Season ††</th>
<th>Maternal education ‡‡</th>
<th>Socioeconomic status ‡§‡§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>1st year</td>
<td>2nd year</td>
<td>Male</td>
<td>Female</td>
<td>Exclusive</td>
<td>Nonexclusive</td>
</tr>
<tr>
<td></td>
<td>crRR</td>
<td>cRR</td>
<td>aRR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylobacter</td>
<td>240</td>
<td>0.73</td>
<td>(187/255)</td>
<td>0.51</td>
<td>(129/252)</td>
<td>0.43</td>
<td>(111/259)</td>
</tr>
<tr>
<td></td>
<td>crRR</td>
<td>0.9 (0.8, 1.1)</td>
<td>1 (1.1, 1.4)</td>
<td>0.9 (0.8, 1.2)</td>
<td>1 (0.9, 1.4)</td>
<td>2.94 (2.5, 3.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>aRR</td>
<td>0.8 (0.7, 0.9)</td>
<td>1 (0.8, 1.1)</td>
<td>1 (0.9, 1.4)</td>
<td>1 (0.9, 1.4)</td>
<td>2.93 (2.4, 3.5)</td>
<td></td>
</tr>
<tr>
<td>Shigella</td>
<td>42</td>
<td>0.8</td>
<td>(25/335)</td>
<td>0.8</td>
<td>(21/336)</td>
<td>0.8</td>
<td>(0.2, 0.7)</td>
</tr>
<tr>
<td>All bacterial diarrhoea</td>
<td>999</td>
<td>2.2</td>
<td>(560/256)</td>
<td>2.1</td>
<td>(534/251)</td>
<td>1.8</td>
<td>(465/260)</td>
</tr>
<tr>
<td></td>
<td>crRR</td>
<td>0.9</td>
<td>(112/155)</td>
<td>1.2</td>
<td>(1.1, 1.4)</td>
<td>1.2 (0.7, 0.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>aRR</td>
<td>0.8</td>
<td>(0.4, 1.4)</td>
<td>1 (0.4, 1.4)</td>
<td>1 (0.4, 1.4)</td>
<td>0.9 (0.6, 1.1)</td>
<td></td>
</tr>
<tr>
<td>All diarrhoea</td>
<td>4,001</td>
<td>4.0</td>
<td>(6,582, 5)</td>
<td>6.3</td>
<td>(1,955, 25)</td>
<td>8.3</td>
<td>(1,885, 25)</td>
</tr>
<tr>
<td></td>
<td>crRR</td>
<td>0.9</td>
<td>(1.5, 1.6)</td>
<td>1.2</td>
<td>(1.1, 1.2)</td>
<td>1.2 (1.0, 1.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>aRR</td>
<td>0.8</td>
<td>(1.1, 1.2)</td>
<td>1 (1.0, 1.2)</td>
<td>1 (0.7, 0.9)</td>
<td>1 (0.8, 1.0)</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05; †p < 0.01; ‡p < 0.001; §: Incidence rate (number of episodes per child-year); #: Crude relative rate; ‡: Adjusted relative risk; adjusted for significant variables that appeared from crude relative risk of each pathogen; **: Crowding classified as greater than or equal to 3.5 persons per bedroom; ††: Warm season defined as months of May to October and cold season as months of November to April; ‡‡‡: Mothers able to read and write classified as literate, otherwise classified as illiterate; §§§: Classified in view of types of luxury items, and kinds of fowls, livestock, and animals owned by household; ‡‡‡‡: All bacterial diarrhea episodes identified as sole or mixed bacterial pathogens (i.e., viral, parasites, and undefined diarrhea cases were excluded from analysis).
<table>
<thead>
<tr>
<th>Hygiene variables</th>
<th>Codes</th>
<th>ETEC</th>
<th>Campylobacter</th>
<th>All bacterial diarrhea</th>
<th>All diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>540</td>
<td>201</td>
<td>852</td>
<td>3,157</td>
</tr>
<tr>
<td></td>
<td>IR §</td>
<td>cRR ¶</td>
<td>aRR #</td>
<td>IR §</td>
<td>cRR ¶</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barriers for birds &amp; animals from eating area</td>
<td>No</td>
<td>1.3</td>
<td>(117/92)</td>
<td>1.00 (0.8, 1.2)</td>
<td>1.00 (0.8, 1.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.3</td>
<td>(423/330)</td>
<td>1.00 (0.8, 1.2)</td>
<td>1.00 (0.8, 1.3)</td>
</tr>
<tr>
<td>Feces in the eating area</td>
<td>Yes</td>
<td>1.5</td>
<td>(42/29)</td>
<td>1.2 (0.9, 1.6)</td>
<td>1.2 (0.9, 1.6)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1.3</td>
<td>(498/393)</td>
<td>1.00 (0.8, 1.2)</td>
<td>1.00 (0.8, 1.3)</td>
</tr>
<tr>
<td>Uncovered garbage in the kitchen</td>
<td>Yes</td>
<td>1.3</td>
<td>(299/226)</td>
<td>1.1 (0.9, 1.3)</td>
<td>1.03 (0.9, 1.2)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1.2</td>
<td>(241/196)</td>
<td>1.00 (0.8, 1.2)</td>
<td>1.00 (0.8, 1.3)</td>
</tr>
<tr>
<td>Presence of garbage containers</td>
<td>Yes</td>
<td>1.4</td>
<td>(173/127)</td>
<td>1.1 (0.9, 1.3)</td>
<td>1.1 (0.9, 1.4)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1.3</td>
<td>(367/295)</td>
<td>1.1 (0.9, 1.3)</td>
<td>1.1 (0.9, 1.4)</td>
</tr>
<tr>
<td>Garbage containers with a lid</td>
<td>No</td>
<td>1.6</td>
<td>(136/84)</td>
<td>1.9 (1.3, 2.7) ‡</td>
<td>1.6 (1.1, 2.3) *</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0.9</td>
<td>(37/43)</td>
<td>1.7 (1.2, 2.3) †</td>
<td>1.4 (1.0, 2.0) *</td>
</tr>
<tr>
<td>Liquid materials in garbage containers</td>
<td>Yes</td>
<td>2.0</td>
<td>(54/27)</td>
<td>1.7 (1.2, 2.3) †</td>
<td>1.4 (1.0, 2.0) *</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>1.2</td>
<td>(119/100)</td>
<td>1.00 (0.8, 1.2)</td>
<td>1.00 (0.8, 1.3)</td>
</tr>
</tbody>
</table>

*: P < 0.05; †: P < 0.01; ‡: P < 0.001; §: Incidence rate (no. of episodes per child-year). ¶: Crude relative rate. #: Adjusted relative risk; each pathogen adjusted for significant socioeconomic, demographic and environmental variables appeared from Table 1, in addition to the repeated observations of the same child, using Poisson regression model and SAS software; **: Included public tap, tube well, canal, and water truck.
<table>
<thead>
<tr>
<th>Hygiene variables</th>
<th>Codes</th>
<th>n</th>
<th>IR §</th>
<th>cRR ¶</th>
<th>aRR #</th>
<th>IR §</th>
<th>cRR ¶</th>
<th>aRR #</th>
<th>IR §</th>
<th>cRR ¶</th>
<th>aRR #</th>
<th>IR §</th>
<th>cRR ¶</th>
<th>aRR #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functioning water tap in washing facility</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>1.1 (80/73)</td>
<td>0.8 (07, 1.1) *</td>
<td>0.7 (06, 1.0) *</td>
<td>0.64 (47/74)</td>
<td>1.5 (1.0, 2.0) *</td>
<td>1.3 (0.9, 1.8)</td>
<td>2.0 (149/73)</td>
<td>1.00 (0.8, 1.2)</td>
<td>1.0 (0.8, 1.1)</td>
<td>7.4 (539/73)</td>
<td>0.98 (0.9, 1.1)</td>
<td>0.88 (0.8, 1.0) *</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>1.3 (460/439)</td>
<td>1</td>
<td>1</td>
<td>0.44 (154/348)</td>
<td>1</td>
<td>1</td>
<td>2.0 (703/349)</td>
<td>1</td>
<td>1</td>
<td>7.5 (2,618/349)</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Fixed raised basin in washing facility</td>
<td></td>
<td>1.2 (136/110)</td>
<td>0.9 (0.8, 1.0)</td>
<td>0.8 (0.7, 1.0)</td>
<td>0.6 (67/110)</td>
<td>1.4 (1.1, 1.9) *</td>
<td>1.3 (1.0, 1.8)</td>
<td>2.1 (228/110)</td>
<td>1.0 (0.9, 1.2)</td>
<td>1.0 (0.8, 1.2)</td>
<td>7.6 (832/110)</td>
<td>1.01 (0.9, 1.1)</td>
<td>0.93 (0.9, 1.0)</td>
<td></td>
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<tr>
<td>No</td>
<td></td>
<td>1.3 (404/312)</td>
<td>1</td>
<td>1</td>
<td>0.43 (134/312)</td>
<td>1</td>
<td>1</td>
<td>2.0 (624/312)</td>
<td>1</td>
<td>1</td>
<td>7.4 (2,325/312)</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Yes</td>
<td></td>
<td>0.5 (3/6)</td>
<td>0.4 (0.1, 1.3)</td>
<td>0.4 (0.1, 1.3)</td>
<td>1.43 (8/6)</td>
<td>3.1 (1.5, 6.3)</td>
<td>2.8 (1.4, 5.7)</td>
<td>2.1 (12/6)</td>
<td>1.1 (0.6, 1.9)</td>
<td>1.1 (0.6, 2.0)</td>
<td>8.9 (50/6)</td>
<td>1.2 (0.9, 1.5)</td>
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<tr>
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<td>1</td>
<td>0.46 (193/416)</td>
<td>1</td>
<td>1</td>
<td>2.0 (840/416)</td>
<td>1</td>
<td>1</td>
<td>7.5 (3,107/416)</td>
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<td></td>
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<tr>
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<td></td>
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<td>0.5 (0.2, 1.1)</td>
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<td>1.16 (10/9)</td>
<td>2.5 (1.3, 4.8)</td>
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<td>0.9 (0.5, 1.5)</td>
<td>9.1 (78/9)</td>
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<tr>
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<td></td>
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<td>1</td>
<td>1</td>
<td>0.46 (191/413)</td>
<td>1</td>
<td>1</td>
<td>2.0 (836/413)</td>
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<td>1</td>
<td>7.4 (3,079/413)</td>
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<td>Functioning water tap</td>
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<td>1.3 (459/348)</td>
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<td>1.3 (1.1, 1.7) *</td>
<td>0.43 (149/348)</td>
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<td>2.2 (160/74)</td>
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<td>7.9 (584/74)</td>
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<tr>
<td>Source of water for the child</td>
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<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Tap inside compound</td>
<td></td>
<td>1.3 (426/323)</td>
<td>1.1 (0.9, 1.4)</td>
<td>1.2 (1.0, 1.5) *</td>
<td>0.41 (132/322)</td>
<td>0.6 (0.4, 0.8)</td>
<td>0.7 (0.6, 1.0)</td>
<td>2.0 (657/323)</td>
<td>0.9 (0.8, 1.0)</td>
<td>0.9 (0.8, 1.0)</td>
<td>7.3 (2,432/323)</td>
<td>0.98 (0.8, 0.9)</td>
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<tr>
<td>Others **</td>
<td></td>
<td>1.1 (114/99)</td>
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<td>1</td>
<td>0.69 (69/100)</td>
<td>1</td>
<td>1</td>
<td>2.2 (215/99)</td>
<td>1</td>
<td>1</td>
<td>8.2 (815/99)</td>
<td>1</td>
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</table>

*: P < 0.05, †: P < 0.01, ‡: P < 0.001, §: Incidence rate (no. of episodes per child-year), ¶: Crude relative rate, #: Adjusted relative risk; each pathogen adjusted for significant socioeconomic, demographic and environmental variables appeared from Table 1, in addition to the repeated observations of the same child, using Poisson regression model and SAS software. **: Included public tap, tube well, canal, and water truck.
In regards to the inappropriate hygiene practices and behaviors related to garbage containers, the presence of garbage containers in the compound (inside and/or outside the house) was associated with an increased risk of Campylobacter diarrhea (aRR 1.4, p < 0.05).

Hygiene behaviors and practices related to washing and/or bathing facilities

Campylobacter diarrhea was the bacterial pathogen most influenced by the hygienic conditions of washing and/or bathing facilities. Among 23 variables studied, Campylobacter diarrhea significantly increased with the presence of a bathing facility in the compound and the presence of feces around or on the floor of bathing facilities (aRR 2.0, p < 0.05 and aRR 2.8, p < 0.01, respectively). Using tap water in washing facilities appeared to decrease the risk ETEC diarrhea (aRR 0.7, p < 0.05) and the risk of infectious diarrhea (aRR 0.88, p < 0.05).

Hygiene behaviors and practices related to water supply and containers

Among the 11 hygiene variables related to the supply and storage conditions of drinking and/or cooking water, municipally piped water was significantly associated with reduced risk of Campylobacter diarrhea (aRR 0.7, p < 0.05).

None of the hygiene behaviors and practices related to sleeping rooms, cooking rooms, previously prepared food, and live flies in houses were found to be significantly associated with the risk of Campylobacter, ETEC, or any bacterial diarrhea.

Discussion

Many studies have indicated bacterial, viral, and parasitic pathogens as the main causes of diarrheal diseases. ETEC, Campylobacter, Shigella, and Salmonella are the most common bacterial diarrheal infections. Bacterial diarrheal infections are mostly spread through contaminated food or water and suboptimal water sanitation. Poor personal hygiene conditions such as maternal hygiene behaviors, and hand washing and cleaning, are often linked to childhood diarrheal illness in developing countries. Animal feces also contain microorganisms that can cause diarrhea [3,7,15].

In this study, hygiene factors associated with eating rooms were identified as high risk factors for diarrhea, especially for Campylobacter diarrhea. The absence of barriers to keep birds and animals outside the eating rooms was found to be significantly associated with the risk of Campylobacter diarrhea (aRR 1.47, p < 0.05), while the presence of animal or human feces in the eating rooms and the presence of feces on the floor or around the walls of the bathrooms were found to be significantly associated with the risk of diarrhea and Campylobacter diarrhea (aRR 1.2, p < 0.05 and aRR 2.8, p < 0.01, respectively). These factors may be considered as indirect indicators of the significant role of the presence of animals and birds inside houses, which contributes to increasing diarrhea, especially Campylobacter-associated diarrhea.

The habit of rearing birds and animals inside the house is widespread in the rural communities of Egypt. In the study’s community, more than 80% of the families reared chickens and ducks, 48% reared pigeons, and 23% reared rabbits inside their houses. In addition, animals and animal feces were observed outside the dining rooms in 90% of the cases where no barriers were used to keep animals and birds outside. Such hygiene conditions represent an excellent environment for Campylobacter growth, frequency, and transmission, especially in the warm season (which was found to be a significant confounder).

This result may be supported by the findings of an observational study conducted in Peru 1990, which showed that children frequently come into contact with poultry feces that lie within the homes and have 3.9 feces-to-mouth episodes within a 12-hour period [15,19]. Many other studies also indicated the association of Campylobacter diarrhea with the consumption of contaminated water or foods, ownership of livestock or poultry, or contact with animals [15]. Domestic birds and companion animals are known as reservoirs for Campylobacter species, and shedding of the bacteria from these reservoirs causes contamination of the environment [20-22]. Strains isolated from human and chickens were phenotypically and genotypically similar, confirming that chickens are an important source of human Campylobacteriosis in developing countries [20,23].

Garbage was another hygiene variable associated with a high risk of bacterial diarrheal diseases. Our initial hypothesis was that the presence of garbage container(s) inside houses would be an indicator of cleanliness and good hygiene practices, leading to reduction of the risk of diseases in children. In the current study, the opposite impact was recorded; high relative risk for Campylobacter diarrhea was observed to be associated with the presence of garbage containers inside houses (aRR 1.4, p < 0.05), and increased relative risk of ETEC was associated with uncovered garbage containers (aRR 1.6, p < 0.05) and
with the observance of liquid materials in the garbage containers (aRR 1.4, p < 0.05). Such findings may reflect the poor hygiene behaviors and practices in dealing with and cleaning garbage containers, thereby increasing the risk of other bacterial pathogens, or the improper containment/maintenance of such garbage cans which serve as a continued source of infection. In fact, we observed that 30% of houses did not empty their garbage containers on a daily basis, and that 63% of houses do not wash their garbage containers on a regular basis. In other words, poor hygiene practices in addition to postponing emptying and washing garbage containers represents a good environment for the fermentation of wastes and rapid growth of bacteria causing diarrhea. Previous studies also indicated the significant role of garbage in acquiring Campylobacter in developing countries [15,20] and the high relative rate of Campylobacter (aRR 2.02, p < 0.01) associated with uncovered garbage [15].

The present study indicated a significant association between water source, storage, and quality and the risk of bacterial diarrhea (ETEC and Campylobacter species). Our results confirm an increased risk for diarrhea with the likelihood of water contamination. This often occurs with the use of non-piped and stored water instead of a municipal source of water for drinking and cooking, and also with poor hygiene conditions of containers (i.e., duration of storage and the possible contact of water with other infected materials) [21]. Our observational study showed that among all cases of bacterial diarrhea, 25% of the families used non-piped water (public tap, tube well, canal, and water truck) as their main source for drinking and cooking. In addition, 3% of the households used stored water and 52% used containers, neglecting basic proper hygiene conditions (i.e., non-daily change of water and non-use of containers with lids or narrow necks to avoid contamination of the stored water). Previous studies have indicated that the lack of piped water in addition to the presence of animals and uncovered garbage are the main risk factors for acquiring Campylobacter in developing countries [15,20]. Overall diarrhea incidence has been noted to be lower in households where water was stored in a container with a tap [24-26].

The present study is an improvement upon potential confounding of associations between hygiene factors and disease risk identified in previous studies. For example, the present study involved a community-based cohort rather than a hospital-based study (that is, investigation of bacterial diarrhea was based on incidence rates rather than prevalence rates), so that past exposure on current susceptibility and seasonality of the disease were considered in the analysis. In addition, previous reports based on cohort studies used only baseline characteristics of the population to identify the risk factors for disease [15]. In the present study, data on household and personal hygiene conditions were updated at regular intervals (every six months) throughout the study and were used to identify disease risk factors based on recent characteristics prior to each episode. Another advantage of the current study is the use of a structured observational questionnaire. Only a few studies of diarrhea epidemiology have used such an approach in the collection of household and individual hygiene information [15,24,27]. The greatest advantage of the structured observational approach is that observation over a longer period of time reduces the degree of reactivity of the observed population, as people become habituated to the observer’s presence. Additionally, variation in the hygiene behavior of each individual from one occasion to the next could be observed and monitored [18,28]. A structured observational approach is extremely expensive and requires substantial investments of time and well-trained staff. However, our study was not without limitations. Viral pathogens other than rotavirus were not tested for; consequently, the incidence of sole bacterial pathogens may have been overestimated.

Less variation in the families’ socioeconomic statuses in the five villages under study may have been a factor in the insignificant impact of SES variables in determining the risk of bacterial diarrhea by different pathogens. Although the independent effect of SES variables on diarrhea incidence was not reported, SES may have acted indirectly through transmission factors, impacting the incidence, as noted by other studies [23,29].

Conclusions
These data highlight some potentially modifiable factors that could reduce the burden of disease in resource-poor settings. Based on data obtained in our study, we can stress two intervention methods to improve the level of hygiene practices and behaviors in these societies. The first is a concerted, focused effort to expand municipal (piped) water supplies to all houses for drinking and cooking, thus reducing the use of potentially contaminated water in containers. The second intervention consists of comprehensive mass media programs to change hygiene behaviors and practices.
However, encouraging behavioral change in any society is a difficult process, as it requires continuous efforts for a long time and a good identification of the target group and targeted hygiene behaviors for improvement [24,30]. In view of this fact, the suggested programs need to be directed to caretakers of children and should concentrate on specific hygiene practices, primarily keeping animals and birds outside the living quarters, proper hand washing after and keeping children away from contact with animal and bird feces, and proper use of garbage containers (covering, emptying, and washing). Such hygiene practices need to be improved in rural villages in Egypt. Perhaps due to the seasonality of infection, reinforced and targeted communications during the summer months may bring value. Increasing the rates and duration of breastfeeding would also support disease mitigation efforts for diarrheal and other infectious diseases.

Other supportive strategies, such as developing a safe and effective bacterial diarrheal disease vaccine for children at an early stage of life, will be helpful to protect children and to reduce the burden of Campylobacter and other important bacterial causes of diarrheal illness in these areas. Further studies are warranted to evaluate hygiene factors for other causes of diarrhea, such as viruses and parasites.

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References

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**Conflict of interests:** No conflict of interests is declared.
Supplementary items

HYGIENE SURVEY

Serial number: __________________________
Week number: __________________________

Village number: ___ ___ ___

House number: ___ ___ ___

Child name: __________________________
Child RID #: __________________________
Mother name: __________________________
Mother RID #: __________________________

Date of visit: __ __ / __ __ / __ __
   d   d   m   m   y   y

Interviewer initials (First, middle, last name) ___ ___ ___
Reviewer initials (First, middle, last name) ___ ___ ___
First data entry technician’s initials
(First, middle, last name) ___ ___ ___
Second data entry technician’s initials
(First, middle, last name) ___ ___ ___
A. SLEEPING ROOM/PLACE

Ask the mother/caretaker to show you the sleeping room/place used by the participating child.

1) Is there a barrier to keep birds and animals out of this sleeping room/place?
   Y = Yes       N = No       Z = Uncertain

2) Are these barriers now in position to keep birds and animals out of the sleeping room/place?
   Y = Yes       N = No       X = No barriers       Z = Uncertain

3) Do you see birds or animals inside the sleeping room/place?
   Y = Yes       N = No       Z = Uncertain

4) Do you see any human or animal feces in the sleeping room/place?
   Y = Yes       N = No       Z = Uncertain

5) Do you see any uncovered garbage in the sleeping room/place?
   Y = Yes       N = No       Z = Uncertain

6) What is the predominant composition of the floor in the sleeping room/place?
   A = Ceramic tiles
   B = Wood
   C = Cement tiles
   D = Cement
   E = Earth and sand
   F = Other, specify: __________________________

7) Is there a bed net for the participating child?
   Y = Yes       N = No       Z = Uncertain

B. EATING ROOM/PLACE

Ask the mother/caretaker to show you the eating room/place used by the participating child.

1) Are there barriers to keep birds and animals out of this eating room/place?
   Y = Yes       N = No       Z = Uncertain

2) Are these barriers now in position to keep birds and animals out of the eating room/place?
   Y = Yes       N = No       X = No barriers       Z = Uncertain

3) Do you see birds or animals inside the eating room/place?
   Y = Yes       N = No       Z = Uncertain

4) Do you see any human or animal feces in the eating room/place?
   Y = Yes       N = No       Z = Uncertain

5) Do you see any uncovered garbage in the eating room/place?
   Y = Yes       N = No       Z = Uncertain
6) What is the predominant composition of the floor in the eating room/place?
   A = Ceramic tiles
   B = Wood
   C = Cement tiles
   D = Cement
   E = Earth and sand
   F = Other, specify: ____________________________

C. COOKING PLACE OR KITCHEN

Ask the mother/caretaker to show you the cooking place or kitchen used by her to prepare food for the participating child.

1) Are there barriers to keep birds and animals out of this cooking place or kitchen?
   Y = Yes  N = No  Z = Uncertain

2) Are these barriers now in position to keep birds and animals out of the cooking place or kitchen?
   Y = Yes  N = No  X = No barriers  Z = Uncertain

3) Do you see birds or animals inside the cooking place or kitchen?
   Y = Yes  N = No  Z = Uncertain

4) Do you see any human or animal feces in the cooking place or kitchen?
   Y = Yes  N = No  Z = Uncertain

5) Do you see any uncovered garbage in the cooking place or kitchen?
   Y = Yes  N = No  Z = Uncertain

6) What is the predominant composition of the floor in the cooking place or kitchen?
   A = Ceramic tiles
   B = Wood
   C = Cement tiles
   D = Cement
   E = Earth and sand
   F = Other, specify: ____________________________

7) Is the kitchen or cooking place completely covered with a ceiling?
   Y = Yes  N = No

8) Is there a working refrigerator in the house?
   Y = Yes  N = No

D. GARBAGE CONTAINER

1) Is there at least one garbage container?
   A = Yes, inside the house
   B = Yes, outside the house
   C = Yes, inside and outside the house
   D = No

   Y = Yes  N = No
If the answer is no skip to section E.

2) Are the garbage containers covered with a lid?
   A = Yes, all are covered
   B = Yes, some but not all are covered
   C = No, none are covered

3) Do any of the garbage containers contain human or animal feces?
   Y = Yes  N = No  Z = Uncertain

4) At this time, do any of the garbage containers contain free liquid in them?
   Y = Yes  N = No  Z = Uncertain

5) Ask the mother/caretaker how often, on average, are the garbage containers emptied:
   A = Daily
   B = Alternate days
   C = Weekly
   D = Less often than weekly
   E = Never

6) Ask the mother/caretaker how often, on average, are the garbage containers washed with water:
   A = Daily
   B = Alternate days
   C = Weekly
   D = Less often than weekly
   E = Never

E. PREVIOUSLY PREPARED FOOD

1) Do you see any previously prepared food (cooked or uncooked)?
   Y = Yes  N = No

If the answer is no skip to section F.

2) Ask the mother if she intends to give the previously prepared food to the participating child?
   Y = Yes  N = No  Z = Uncertain

If the answer is no skip to section F.

3) Ask the mother: Has all of the food been prepared today?
   Y = Yes  N = No

4) From your observation, how much of the food is covered?
   A = All is covered
   B = Some but not all is covered
   C = None is covered
5) From your observation, how much of the food is kept above floor level?
   A = All is kept above the floor level
   B = Some but not all is kept above floor level
   C = None is kept above floor level (all the food is on the floor level)

F. WASHING FACILITIES

Ask the mother/caretaker to show you where, in the house, she washes her hands.

1) Observe if the following are available:
   a) Functioning water tap
      Y = Yes N = No
   b) Drain for water
      Y = Yes N = No
   c) Fixed raised bowel or basin
      Y = Yes N = No
   d) Bowl(s) on the floor
      Y = Yes N = No
   e) Soap for hand washing
      Y = Yes N = No
   f) Ash for hand washing
      Y = Yes N = No
   g) Towel or rag for drying hands
      Y = Yes N = No

2) Do you notice a container of used water in the washing area?
   A = Yes, container on the floor
   B = Yes, container not on the floor
   C = No container of used water

G. BATHROOM (marahid)

1) Is there a bathroom in the house?
   Y = Yes N = No

If the answer is no, skip to question 11.
If the answer is yes, answer the questions about the bathroom used by the mother/caretaker of the participating child.

2) Where is the bathroom?
   A = Inside the house
   B = Inside the compound but outside the house

3) What kind of a toilet or latrine do you see?
   A = Modern
   B = Local with flush
   C = Local without flush
   D = Pit
   E = Other, specify: ____________________________

4) Ask the mother/caretaker: Where does latrine drain?
   A = Sewage system
   B = Sealed pit
   C = Unsealed pit
   D = Drains to environment

5) Inside the bathroom, do you see any feces on the floor?
   Y = Yes N = No Z = Uncertain
6) Are there any human or animal feces outside the bathroom, but within 3 paces of the outside wall of the bathroom?  
   Y = Yes  N = No  Z = Uncertain

7) Inside the bathroom, do you see any puddling on the floor?  
   Y = Yes  N = No  Z = Uncertain

8) Do you see any puddling outside the bathroom, but within 3 paces of the outside wall of the bathroom?  
   Y = Yes  N = No  Z = Uncertain

9) Is there a place for hand washing in the bathroom or within 10 paces of the bathroom’s entrance?  
   A = Yes, inside the bathroom  
   B = Yes, outside (within one pace of) the bathroom  
   C = Yes, outside within 10 paces of bathroom entrance  
   D = No, not seen within 10 paces of bathroom entrance

10) What is the predominant composition of the bathroom floor (excluding the squatting slab)?  
    A = Ceramic tiles  
    B = Wood  
    C = Cement tiles  
    D = Cement  
    E = Earth and sand  
    F = Other, specify: __________________________

11) Do you see any potties (asria) in the bathroom or elsewhere in the house?  
    Y = Yes  N = No

12) Are any of the potties (asria) used by the participating child?  
    Y = Yes  N = No  X = No potty  Z = Uncertain

H. BATHING FACILITY

1) Is there a bathing facility in the compound?  
   Y = Yes  N = No

If the answer is no, skip to section I.  
If the answer is yes, answer the questions about the bathing facility used by the mother/caretaker of the participating child.

2) Where is the bathing facility?  
   A = Inside the house  
   B = Inside the compound but outside the house

3) What kind of bathing facility is it?  
   A = Modern fitment attached to water supply  
   B = Bowl or bucket, and dipper  
   C = Other, specify: __________________________
I. WATER SOURCES AND CONTAINERS

1) Is there a functioning water tap in the compound?
   A = Yes, inside the house
   B = Yes, outside the house
   C = No water tap in the compound

2) What is the source of water that she uses for drinking water for the participating child?
   A = Tap in compound
   B = Public tap
   C = Tube well
   D = Canal
   E = Water truck
   F = Other, specify: _____________________________

3) What is the source of water that she uses for cooking for the participating child?
   A = Tap in compound
   B = Public tap
   C = Tube well
   D = Canal
   E = Water truck
   F = Other, specify: _____________________________

4) Ask the mother/caretaker whether the household uses container(s) to store water for drinking.
   Y = Yes  N = No

   If the answer is no, skip to question 8.

5) For the water container(s) the household uses for drinking water, indicate whether the container has a narrow neck so that the hand cannot touch the water in the container:
   A = All of the containers have a narrow neck
   B = Some but not all of the containers have a narrow neck
   C = None of the containers have a narrow neck

6) For the water container(s) the household uses for drinking water, indicate whether a dipper with a long handle is seen in the immediate vicinity of the container:
   A = All of the containers have a dipper
   B = Some but not all of the containers have a dipper
   C = None of the containers have a dipper

7) Ask the mother/caretaker how often, on average, the water in the container(s) used for drinking water is changed:
   A = Daily
   B = Alternate days
   C = Weekly
   D = Less often than weekly
   E = Never

8) Ask the mother/caretaker whether the household uses container(s) to store water other than those containers used for drinking water.
   Y = Yes  N = No
If the answer is no, skip to section J.

9) For the household water container(s) **not used for drinking** water, indicate whether the container has a narrow neck so that the hand cannot touch the water in the container:
   A = All of the containers have a narrow neck
   B = Some but not all of the containers have a narrow neck
   C = None of the containers have a narrow neck

10) For the household water container(s) **not used for drinking** water, indicate whether a dipper with a long handle is seen in the immediate vicinity of the container:
    A = All of the containers have a dipper
    B = Some but not all of the containers have a dipper
    C = None of the containers have a dipper

11) Ask the mother/caretaker how often, on average, the water in the container(s) **not used for drinking** water is changed:
    A = Daily
    B = Alternate days
    C = Weekly
    D = Less often than weekly
    E = Never

**J. FLIES**

1) Do you notice any live flies?
   a) In the cooking area: Y = Yes  N = No  Z = Uncertain
   b) In the eating area: Y = Yes  N = No  Z = Uncertain
   c) In the sleeping area: Y = Yes  N = No  Z = Uncertain
   d) In the open courtyard: Y = Yes  N = No  Z = Uncertain
   e) Elsewhere in house: Y = Yes  N = No  Z = Uncertain

2) Is the participating child inside the house?
   Y = Yes  N = No

3) Do you notice any live flies around the child’s face?
   Y = Yes  N = No  X = Child not in house