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Hygiene intervention reduces contamination of weaning food in Bangladesh

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Abstract

OBJECTIVE This study was conducted to measure the impact of a hygiene intervention on the contamination of weaning food in Bangladesh.

METHODS Sixty households were selected: 30 study and 30 control households. Samples of weaning food were collected from all the 60 households at baseline and examined for faecal coliforms (FC), faecal streptococci (FS) and *Clostridium perfringens* (CP) following standard procedures. After cooking, food samples were collected on three occasions before feeding. Following Hazard Analysis Critical Control Point (HACCP) procedures, critical control points were determined. The mothers in the 30 study households were then trained for 4 weeks in how to attain the control point conditions. Then, again the food samples were collected and analysed.

RESULTS At baseline, weaning foods from study and control households were heavily contaminated with FC and FS. The FC and FS counts were 1.84 $\log_{10}$ and 1.92 $\log_{10}$ colony-forming unit (cfu)/g, respectively, in the study households, and 0.86 $\log_{10}$ and 1.33 $\log_{10}$ cfu/g, respectively, in the control households in the first feeding. After the intervention, the FC and FS counts in study households had dropped to 0.10 $\log_{10}$ and 0.09 $\log_{10}$ cfu/g, respectively, a statistically significant reduction ($P < 0.001$). Monitoring the sustainability of the behaviour change after 3 months showed that the mothers were maintaining food hygiene.

CONCLUSIONS A hygiene intervention following the HACCP approach reduced the weaning food contamination significantly. Awareness building among mothers about weaning food hygiene could be an important intervention for preventing weaning food–related diarrhoea in Bangladesh.

keywords weaning food, hazard analysis critical control point, hygiene intervention, diarrhoea, Bangladesh

Introduction

Worldwide, it is estimated that 1400 million episodes of diarrhoea occur annually in children under the age of 5 years, of which over 3 million such children died due to diarrhoea in 1990 (Mortajemi *et al.* 1993). The importance of food safety in the prevention of diarrhoeal diseases is often overlooked. The strategies for prevention of diarrhoeal diseases are often limited to promotion of breastfeeding or improving water supply and sanitation, neglecting the need to educate food handlers, particularly mothers, in food safety. Recent evidence suggests that weaning foods prepared in unhygienic conditions can be highly contaminated with diarrhoea pathogens and are a major cause of diarrhoea and associated malnutrition (Henry *et al.* 1990; Hendrick & Badruddin 1994; Ehiri & Prowse 1999; Oluwanfemi & Ibeh 2011; Touré *et al.* 2011).

In the USA, 76 million episodes of illness, 325 000 hospitalisations and 5000 deaths occur each year due to food-borne diseases (Mead *et al.* 1999). Adak *et al.* (2005) estimated the corresponding totals for the UK: 2 366 000 cases, 21 138 hospitalisations and 718 deaths. The level of faecal contamination is higher in weaning foods than in drinking water (Esrey & Feachem 1989; Mortajemi *et al.* 1993). Rowland *et al.* (1978) discovered that traditional gruels used in The Gambia to supplement

breast milk were usually contaminated with potentially pathogenic microorganisms, and such supplements were important factors in weaning-related diarrhoea. In low-income countries, 70% of episodes of diarrhoea are due to unhygienic preparation of weaning foods (Esrey 1990; Mortajemi *et al.* 1993; Keusch *et al.* 2006). Thus, foods could play a significant role in diarrhoeal disease causation, but more studies are needed to fully understand the epidemiology of food-borne infections and the burden of diarrhoea attributable to food contamination.

Microbial contamination is an indicator of unsafe handling of food, making it a vehicle for transmission of enteric pathogens (WHO 1993a). Contaminated water also contaminates food. There are two common practices in food handling that increase the risk of food-borne diseases: the first is storage of food for several hours before consumption at temperatures that favour growth of pathogens and/or formation of bacterial toxins; the second is insufficient cooking or reheating of preserved food (WHO 1993a). Microbial contamination of food invariably makes the weaning period most hazardous, particularly with respect to diarrhoeal diseases.

Diarrhoeal disease is a major source of morbidity and mortality in Bangladesh, causing 75 million episodes and 110 000 deaths every year (Ahmed 1999; Islam *et al.* 2011). Numerous studies in Bangladesh have shown the importance of food-borne transmission of enteric pathogens. Stanton and Clemens (1987) showed a positive association between frequency of hand-washing prior to food preparation and incidence of diarrhoea among children consuming the food, frequency of hand-washing being indicative of the level of hygiene practice. Black *et al.* (1982) reported an increase in contamination of food at the household level with temperature and duration of storage. Henry *et al.* (1990) observed an increase in coliform count when there was a delay of more than 4 h between preparation and consumption of weaning food. Islam *et al.* (1993) found that the count of *Shigella flexneri* in boiled rice, lentil soup, milk, mashed potato, fish, beef, etc. increased by 2–3 logs within 6 h. This study demonstrated that Bangladeshi food if contaminated can support the growth of diarrhoea-causing bacteria, especially in the absence of hygienic food practice and refrigeration.

Contamination of food is a major cause of diarrhoeal diseases in both the developed and developing world. Recently Touré *et al.* (2011) Touré *et al.* (2012) found that an intervention developed using a Hazard Analysis Critical Control Point (HACCP)-type approach was very effective in reducing contamination of home-cooked weaning food in peri-urban Mali. This study sought to replicate that study in the very different setting of rural Bangladesh and thus to find out whether such results could be achieved in other low-income countries.

Methods

Study sites

The study was conducted at Matlab, a subdistrict and a rural area of Bangladesh, for 7 months from October 2010 to April 2011. Matlab is located about 55 km south-east of Dhaka, the capital city of Bangladesh. In Matlab, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) has been conducting a demographic surveillance for more than 40 years in 200 000 population. In 1963, the ICDDR,B, formerly Cholera Research Laboratory, implemented a health research programme in Matlab, Bangladesh. The Health and Demographic Surveillance System (HDSS), formerly Demographic Surveillance System (DSS), is one of the major components of this field programme. Every household in Matlab HDSS area has a current identification (CID) and registered identification (RID) numbers. Using these unique numbers, the socioeconomic variables, for example age, sex, migration in, migration out, family income, educational status, morbidity and mortality due to various diseases, can be obtained from the database for more than 40 years. The Matlab HDSS is recognised worldwide as one of the longest continuing demographic surveillance sites in a low-income country.

Study households

Before randomisation of the study households, information was collected from the Matlab HDSS database about the presence of children who were taking weaning foods such as *Suzi* and *Khichuri*. Then, a list was prepared based on the collected information. From the surveillance data, 500 households with children aged 6–18 months were screened. Among these screened households, 72 fed the children *Khichuri* and *Suzi* as weaning foods. Of these 72 households, 60 were randomly selected for study. Thirty households were allocated to the study group and 30 to the control group. Both groups had similar medium to low socioeconomic status. After determining the critical control points (CCPs), the field workers provided training to the mothers how to avoid contamination of weaning food during storage and feeding of the child.

Three female field workers were engaged to follow the mothers. Each was in charge of 20 mothers (10 intervention and 10 control households) and also coordinated food sampling in those households.

Identification and monitoring of critical control points

A CCP is a point, step or procedure at which a significant hazard occurs in food preparation and handling and at which control can be applied to prevent, eliminate or reduce the hazard to an acceptable level (Ehiri *et al.* 2001). CCPs were identified through examination of the processing steps listed in the flow diagram (Figure 1). The HACCP decision algorithm was applied to each step of food preparation and handling, to determine whether it should be considered as a CCP (National Sanitation Foundation 2006). CCP determination involves identifying and characterising the hazards, the control measures and the processing steps where control is applied. The main concern was to destroy faecal bacteria in the weaning foods. For each weaning food, 4 CCPs were identified (Figure 1): (i) cooking, (ii) reheating before second and third feedings, (iii) cooling before child feeding for first, second and third time and (iv) feeding child for first, second and third time. Accordingly, the field workers trained the mothers of the intervention group during 4 weeks, assessed their knowledge, observed them during the whole process, noted deviations and recommended the following corrective measures: (i) washing hands with safe water (water free from harmful microorganisms and substances) and soap before starting meal preparation or feeding a child, after cleaning a child's bottom and after using a latrine; (ii) using safe water to wash utensils and prepare food; (iii) cooking and reheating foods until boiling; and (iv) covering the food with a lid during storage. After 4 weeks of training, food samples were again collected from both intervention and control households and examined in the Environmental Microbiology Laboratory of ICDDR,B.

Three rounds of samples were collected from the study households. The first and second rounds were collected during the baseline survey and just after intervention. The third round was collected 3 months later, and examinations were conducted to assess the persistence of behaviour change in the intervention group. At this stage, 15 mothers from the intervention group were observed during preparation of the sampled foods and 15 were not observed, in order to assess the influence of the field workers' presence on the mothers' behaviour. The mothers had told the field workers the approximate time of feedings of the non-observant group previously; therefore, the field workers visited and collected the weaning foods just before feeding the child.

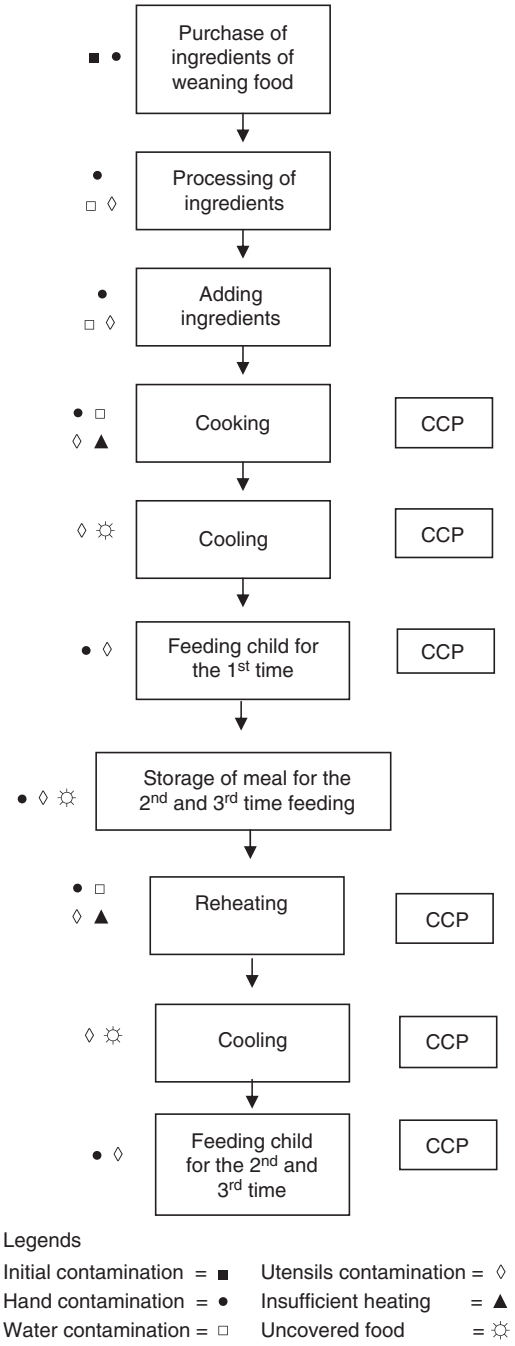

Figure 1 Flow diagram of determination of critical control points (CCPs) of weaning foods following hazard analysis critical control point procedure.

Monitoring of physical and chemical properties of food and water

Weaning foods and water were collected from all the households in both groups. The weaning foods included *Suzi* (semolina; coarse flour of wheat cooked with milk and sugar) and *Khichuri* (rice cooked with lentils and vegetables). The food samples were collected from the weaning

food that was cooked on that particular day of sampling. The weaning food is cooked early in the morning and after the first feeding, and the remainder is stored for the second and third feedings, later in the day. The physical and chemical parameters of the food were measured following the procedures described elsewhere (Islam *et al.* 2000; Touré *et al.* 2011 Touré *et al.* 2012). The temperature of the weaning foods was measured using a thermometer (76 mm Immersion, N2 Filled, Strengthened; Zeal, UK). The pH of the food was measured using a pH meter used for food (Orion 2 Star pH Benchtop; Thermo Scientific, Singapore). The food samples (50 g each) were collected just before feeding using the same spoon used for the feeding of the child and kept in a sterile whirl pack and transported to the laboratory in an insulated cool box with ice packs maintaining the temperature at 4–8 °C. The samples were processed within 6 h of collection.

Microbiological examination of food

Ten grams of weaning food was taken, and 90 ml of sterile normal saline was added. Then, the sample was homogenised using a homogeniser (StedFastTM Stirrer, Model 300; Fisher Scientific). Serial 10-fold dilutions of the homogenised food were then prepared, and 100 μl of the homogenised food was inoculated onto duplicate plates of membrane faecal coliform (mFC) and KF-streptococcus agar media following the spread plate technique to observe the reproducibility (Islam *et al.* 1994 Islam *et al.* 2000). In the case of *C. perfringen*s, 500 μl of food sample was inoculated onto modified *Clostridium perfringens* (mCP) agar media (Sartory *et al.* 1998).

Microbiological examination of water

The water samples were tested for faecal coliforms (FC) and faecal streptococci (FS) following procedures described elsewhere. In brief, for FC and FS, 100 ml water samples were filtered through 0.22μm-pore-size membrane filters (Millipore Corp., Bedford, MA, USA), and the filters were placed on mFC and KF-streptococcus agar plates. All the mFC plates were incubated at 44 °C for 18–24 h for FC. Then, the characteristic blue colonies were counted as FC. All the KF-streptococcus agar plates were incubated at 37 °C for 48 h, and the characteristic light and dark red colonies were counted as FS following standard procedures (American Public Health Association 1998; Islam *et al.* 2007).

For *C. perfringens*, 100 ml of water sample was passed through a Millipore membrane filter, and this filter paper was placed onto mCP medium. This mCP medium was then incubated in an anaerobic jar at 44 °C for 24 h. The yellow colonies were counted as *C. perfringens*. Then, the colonies were further tested by exposing them to ammonium hydroxide following standard procedures (Bisson & Cabelli 1979). The counts of FC, FS and CP were expressed as cfu per 100 ml for water samples and per gram for food samples.

Data analysis

We estimated the means of the log-transformed bacterial counts and compared the means between control and intervention group as well as in between baseline and follow-up. We used *t*-test to test the equality of two means to see whether the difference in means between groups was by chance.

Results

Contamination of weaning foods in baseline and subsequent feedings

The contamination of weaning food was shown by the counts of FC in the study households in the first, second and third feedings. In the baseline survey, these were 1.84 $\log_{10}$, 2.51 $\log_{10}$ and 2.66 $\log_{10}$ cfu/g, respectively; after intervention, they were reduced to 0.10 $\log_{10}$, 0.0 and 0.0 cfu/g, respectively (Figure 2). The reduction in FC counts between the baseline survey and after the intervention was statistically significant ($P < 0.001$).

In control households, the counts of FC in the baseline survey in the first, second and third feedings were 0.86 $\log_{10}$, 1.84 $\log_{10}$ and 2.17 $\log_{10}$ cfu/g, respectively. After 1 month without any intervention, the corresponding counts were 1.42 $\log_{10}$, 1.64 $\log_{10}$ and 1.61 $\log_{10}$ cfu/g, respectively. Thus, there was no significant reduction in FC counts in the control households.

A similar trend was observed for FS counts in the control households, which were 1.64 $\log_{10}$, 2.06 $\log_{10}$ and 2.22 $\log_{10}$ cfu/g in the first, second and third feedings, respectively. The levels of contamination of weaning foods by *C. perfringens* in the first, second and third feedings were 0.06 $\log_{10}$, 0.02 $\log_{10}$ and 0.05 $\log_{10}$ cfu/g, respectively, in the baseline survey. After intervention, the counts in all the feedings were reduced to 0.0 cfu/g. However, in the control households, the contamination by *C. perfringens* persisted in all the feedings, although the contamination levels were very low throughout.

Contamination of source and POU water

The count of FC in the source water (tubewell water) was 0.55 $\log_{10}$ cfu/100 ml in the baseline survey, reducing to

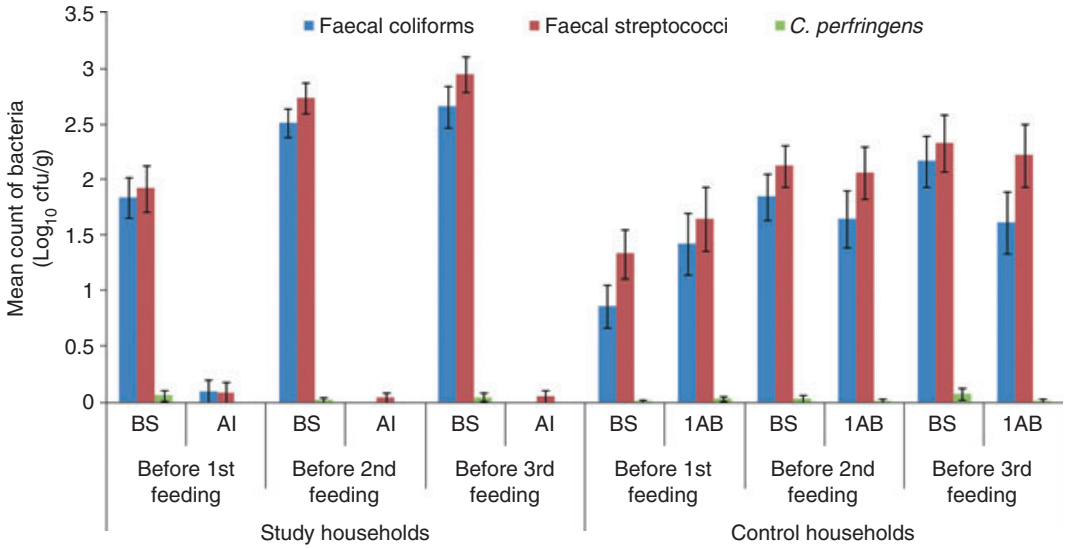

Figure 2 Contamination of weaning foods in baseline and subsequent feedings in study and control households. BS, baseline; AI, after intervention; 1AB, 1 month after baseline.

0.28 $\log_{10}$ cfu/100 ml after intervention in the study households (Figure 3), a statistically significant reduction ($P < 0.01$). In the case of water at point of use (POU), the FC counts were reduced from 2.83 $\log_{10}$ to 0.28 $\log_{10}$ cfu/100 ml after intervention in the study households, which is highly significant ($P < 0.001$). A similar trend was also observed in the reduction in FS in the water used, both at the source and at POU. The counts of FC, FS and *C. perfringens*, both in source and POU water, were also reduced in the control households when the samples were collected. However, the reduction in bacterial counts in the control households was less than in the study households.

Contamination of weaning foods in presence and absence of field workers in study households

Figure 4 shows the contamination in the 15 households that were observed 3 months after intervention to find out the influence of the presence of a field worker during food preparation. Results showed that at baseline the counts of FC, FS and CP were 1.69 $\log_{10}$, 1.78 $\log_{10}$ and 0.04 $\log_{10}$ cfu/g, respectively, in the first feeding. There was a 100% reduction in FC, FS and CP, 1 month and 3 months after intervention in the first feeding. In the second and third feedings, the counts of FS after 1 month were 0.09 $\log_{10}$ and 0.11 $\log_{10}$ cfu/g, substantially and significantly reduced from baseline. There were 100% reductions in all the bacterial counts in the samples collected after 3 months in the second and third feedings of the observed study households.

In non-observed study households, the contamination of households by FC, FS and CP was 1.98 $\log_{10}$, 2.05 $\log_{10}$ and 0.08 $\log_{10}$ cfu/g, respectively, in the first feedings in the baseline survey, which dropped to 0.20 $\log_{10}$, 0.18 $\log_{10}$ and 0.0 $\log_{10}$ cfu/g, respectively, 1 month after the intervention. After 3 months in the intervention households and in the absence of the field worker, the counts of FC and FS increased to 0.33 $\log_{10}$ and 0.36 $\log_{10}$ cfu/g, respectively. Similar results were also observed during second and third feedings. The counts of CP in the first and third feedings were 0.08 $\log_{10}$ and 0.06 $\log_{10}$ cfu/g, respectively, at baseline, but no contamination was observed in the samples collected one and 3 months after the intervention.

Contamination of source and POU water in study households

The contamination of source water in the observed study households was reduced in the samples collected 1 and 3 months after the baseline survey. The FC and FS counts in source water after 3 months in the observed households and in the presence of a field worker were 0.18 $\log_{10}$ and 0.22 $\log_{10}$ cfu/100 ml, respectively, but for non-observed households, the counts were 0.40 $\log_{10}$ and 0.28 $\log_{10}$ cfu/100 ml, respectively, higher than the observed households (Figure 5), which was not significant ($P = 0.68$) for FS but significant ($P = 0.04$) for FC counts. In POU water, the FC and FS counts after 3 months were 0.14 $\log_{10}$ and 0.13 $\log_{10}$ cfu/100 ml in the observed

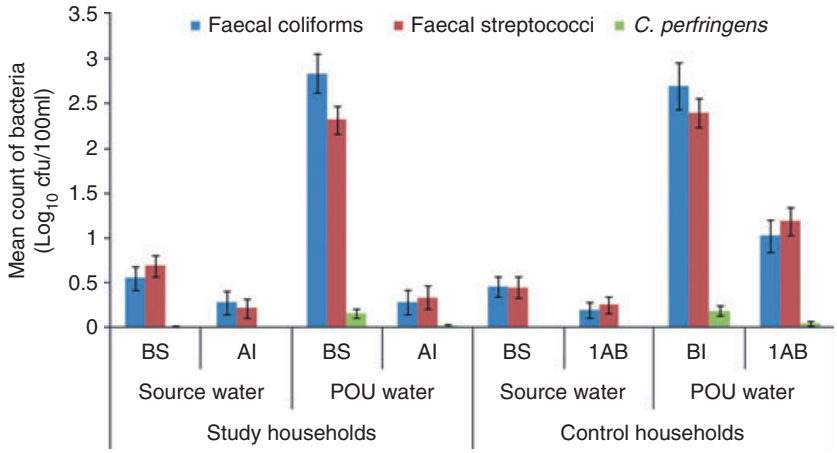

Figure 3 Contamination of source and point of use water in study and control households. BS, baseline; AI, after intervention; 1AB, 1 month after baseline.

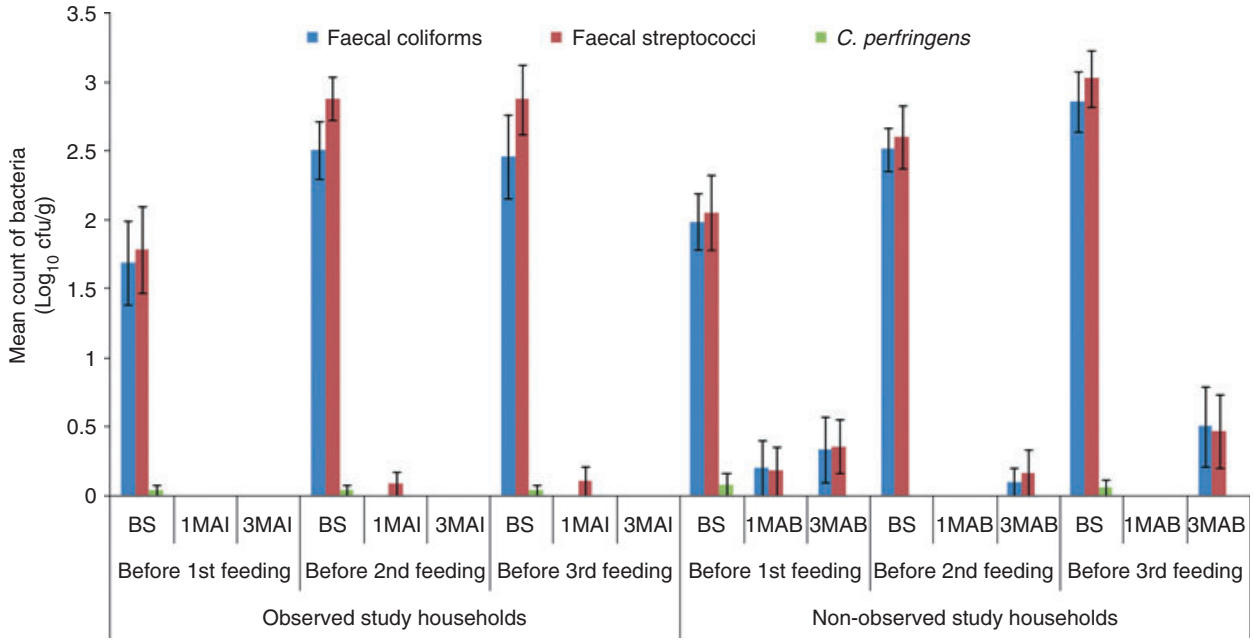

Figure 4 Contamination of weaning foods in presence and absence of field workers in study households. BS, baseline; 1MAI, 1 month after intervention; 3MAI, 3 months after intervention; 1MAB, 1 month after baseline; 3MAB, 3 months after baseline.

households but 0.43 $\log_{10}$ and 0.42 $\log_{10}$ cfu/100 ml, respectively, in non-observed households. Thus, the counts in the non-observed group were nearly three times higher than in the observed group.

Temperature and pH of weaning foods

The temperature in the food just after cooking was almost the same (97.6 ± 2.4–98.7 ± 1.9 °C) in the study and control households (Table 1). The temperatures of weaning foods in the study households in the first, second and third feedings were 41.8 ± 3.8, 39.0 ± 4.7 and 39.0 ± 5.6 °C, respectively, before intervention. In control households, the temperatures during the first, second and third feedings were 40.4 ± 3.7, 37.8 ± 5.0 and 36.6 ± 5.7 °C, respectively, during baseline survey. However, after intervention, the temperatures of weaning foods in the study households were 43.4 ± 4.5,

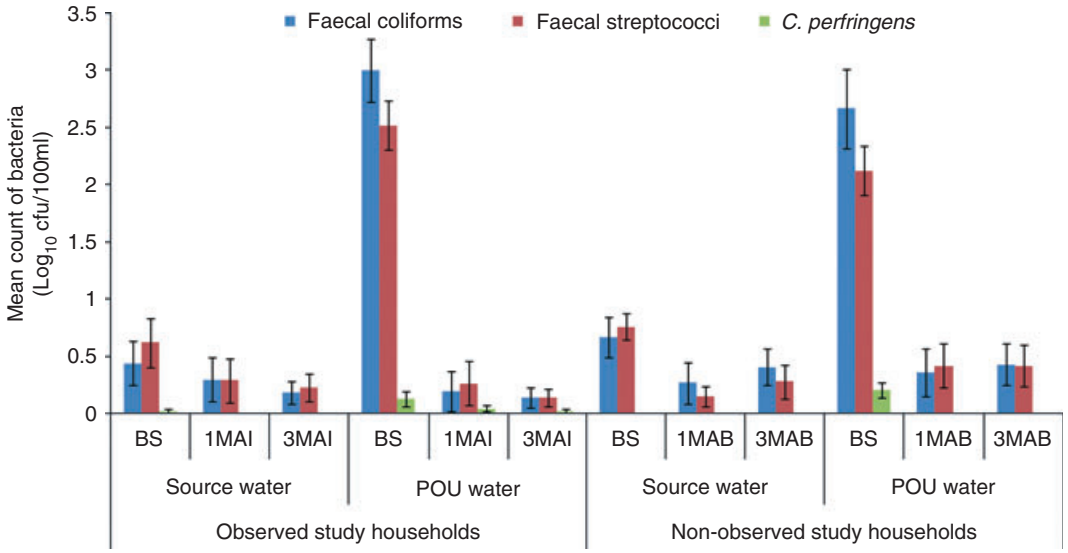

Figure 5 Contamination of source and point of use water in study households. BS, baseline; 1MAI, 1 month after intervention; 3MAI, 3 months after intervention; 1MAB, 1 month after baseline; 3MAB, 3 months after baseline.

Table 1 Temperature and pH of weaning foods

		Temperature (°C)				pH		
Households	Time interval	Just after cooking	Before 1st feeding	Before 2nd feeding	Before 3rd feeding	Before 1st feeding	Before 2nd feeding	Before 3rd feeding
Study	Baseline	97.6 ± 2.4	41.8 ± 3.8	39.0 ± 4.7	39.0 ± 5.6	7.2 ± 0.3	7.1 ± 0.3	7.1 ± 0.3
	After intervention	98.7 ± 2.0	43.4 ± 4.5	42.9 ± 5.0	42.1 ± 5.0	7.2 ± 0.7	7.3 ± 0.8	7.3 ± 0.8
Control	Baseline	97.6 ± 2.6	40.4 ± 3.7	37.8 ± 5.0	36.6 ± 5.7	7.2 ± 0.3	7.1 ± 0.3	7.1 ± 0.3
	1 month after baseline	97.7 ± 2.6	37.4 ± 5.6	30.5 ± 7.1	27.4 ± 6.8	6.9 ± 0.6	7.0 ± 0.5	6.9 ± 0.6

42.9 ± 5.0 and 42.1 ± 5.0 °C in the first, second and third feedings, respectively, after reheating (Table 1). The temperature in study households increased after intervention, but in control households, there was a decrease in temperature as the control households did not practice reheating. There was not much difference in the pH of the weaning foods between study and control households, or before and after the intervention.

Discussion

The baseline survey revealed that there is substantial and widespread contamination of weaning food in Matlab, which is likely to contribute to the food-related diarrhoea in children in Bangladesh (Figure 1). Our results support the findings of other studies that observed that most of the diarrhoea in developing countries is due to preparation of weaning foods under unhygienic conditions (Mortajemi *et al.* 1993; Keusch *et al.* 2006). However, more studies are needed to fully understand the burden of diarrhoea attributable to weaning food contamination.

The intervention following HACCP procedures substantially reduced faecal bacteria. The difference in both FC and FS in weaning foods before and after intervention is statistically significant ($P < 0.001$) (Figure 1), clearly indicating that preparation of weaning food under proper hygienic conditions can reduce its contamination and hence diarrhoea in children in Bangladesh. It also shows that such conditions are achievable, even in poor households in rural Bangladesh.

The water used for washing utensils (e.g. the spoon, the pot in which the weaning food is stored or the small bowl used to keep the food just before feeding) was contaminated by FC, by FS and, to a lesser extent, by CP

(Figure 3). The contaminated water was also used by the mother for washing her hands, which could contaminate them. However, after the intervention, the counts of all the bacteria were reduced significantly ($P < 0.001$) in water at point of use (POU) (Figure 3). All these results provide evidence that the hygiene intervention worked well and reduced the contamination of the weaning foods.

Critical control points were determined following HACCP-type procedures and used to develop the intervention provided to the mothers. Although the HACCP concept was originally used in food processing plants, it can be applied to food preparation at home to ensure the safety of weaning foods (Bryan 1992; Codex Alimentarius Commission 1993; WHO 1993b WHO 1997). The strategy can be used to identify food-borne hazards and assess related risks, and to facilitate the design of effective preventive mechanisms (Moy *et al.* 1997).

The results 3 months after intervention showed that the hygiene intervention can be sustainable because the mothers kept practising it even when there was no follow-up by the field workers for 3 months. However, the presence of field workers during food preparation, storage and feeding did have some impact as the counts of both FC and FS were smaller in study households when field workers were present. To maintain better hygiene practices in the non-observed group, it needs strong motivation.

In study households, the temperature of the food was always higher after the intervention than before as the mothers properly reheated the food before feeding the child; in control households, it was always lower (Table 1). In the control households, the foods were not properly reheated before the second and third feedings of the child, which would explain the failure to reduce contamination. Reheating clearly played an important role in reducing weaning food contamination. The pH of weaning foods used in Bangladesh did not differ significantly between the study and control households, remaining in the range 6.9–7.3. Therefore, pH does not appear to play any significant role in multiplication of bacteria in weaning food in Bangladesh.

This study has demonstrated that a hygiene intervention following a HACCP-type approach can reduce the contamination of weaning food and household water in Bangladesh substantially. This might have an impact on prevention of weaning food–related diarrhoea in Bangladesh. The usefulness of the HACCP approach in the promotion of hygiene of weaning foods lies not in the establishment of new risk factors, but in the determination of points in the food preparation–handling–serving chain which are critical to safety, thus facilitating appropriate targeting of educational messages, and of prevention efforts and resources (Ehiri & Prowse 1999). Application of the HACCP strategy in the design of weaning food hygiene promotion interventions has the potential to contribute significantly in this regard, thus facilitating a more pragmatic preventive approach rather than curative remedies applied after disease has occurred.

The results of this study are very encouraging. A trial is now required involving a larger population for a longer time, to find out whether they are reproducible and applicable on a larger scale and then to assess the impact of the intervention on diarrhoeal disease.

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