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Contamination of weaning foods and transmission of enterotoxigenic *Escherichia coli* diarrhoea in children in rural Bangladesh

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Summary

In longitudinal studies of infectious diseases and nutrition in Bangladesh, we determined the degree of bacterial contamination of traditional weaning foods and evaluated the role of these foods in the transmission of diarrhoeal diseases. 41% of samples of food items fed to weaning aged children contained *Escherichia coli*; these organisms were used as indicators of faecal contamination. Milk and foods prepared particularly for infants were more frequently and heavily contaminated with *E. coli* than was boiled rice, and *E. coli* levels were found to be related to the storage of cooked foods at high environmental temperatures. 50% of drinking water specimens also contained *E. coli* but colony counts were approximately 10-fold [illegible] than in food specimens. The proportion of a [illegible] food samples that contained *E. coli* was [illegible]ficantly related to the child's annual incidence of diarrhoea associated with enterotoxigenic *E. coli*. This observation underscores the importance of seeking locally available foods that are hygienic as well as nutritious to supplement the diets of breastfeeding children in developing countries.

Introduction

Growth retardation and malnutrition are common problems among children in developing countries (MATA, 1978; BROWN *et al.*, in press). Studies in several areas of the world have demonstrated that the impaired growth rates of children in these countries can be partly explained by the high prevalence of diarrhoeal diseases (MARTORELL *et al.*, 1975; ROWLAND *et al.*, 1977). Since standards of personal hygiene and public sanitation are low in many communities in developing countries, contamination of infant foods with pathogenic micro-organisms may be an important source of infectious diarrhoea (GORDON *et al.*, 1963; BROWN, 1978). This diarrhoea could impair the growth of infants and young children. Indeed, bacterial contamination of weaning foods has been observed in studies in one pre-industrial setting in rural West Africa (ROWLAND *et al.*, 1978; BARRELL & ROWLAND, 1979). However, a relationship between the contamination of infant food and the risk of diarrhoea has not been demonstrated.

In most traditional communities in the developing world, breastfeeding is practiced almost universally. Because of the considerations noted above, some workers have suggested delaying the introduction of supplementary foods as long as possible (ANON., 1977). However, in studies in Bangladesh, we found that the intake of breast milk alone was inadequate to satisfy the nutrient requirements recommended by the Food and Agriculture Organization and the World Health Organization (BROWN et al., in press; WORLD HEALTH ORGANIZATION, 1973). Indeed, the growth rate of infants was already faltering compared with an international reference population before the children were six months old (BROWN *et al.*, in press; National Centre for Health Statistics, 1977). In those studies, we monitored morbidity from infectious diseases during alternate-day surveillance in two rural villages in Bangladesh, as well as the dietary intakes and growth patterns of infants and pre school-aged children (BROWN *et al.*, in press; BROWN *et al.*, in press; BLACK *et al.*, 1982a; BLACK *et al.*, 1982b). These studies provided the opportunity to measure the degree of bacterial contamination of traditional weaning foods and to determine if those foods could be directly implicated in the transmission of diarrhoeal disease.

Patients and Methods

The study was conducted in two villages in the Matlab field research area of the International Centre for Diarrhoeal Diseases Research, Bangladesh (formerly the Cholera Research Laboratory). The field area is in the low-lying deltaic plain of Bangladesh, 45 km south-east of the capital, Dacca. Longitudinal studies were initiated in March 1978; previous reports have described the study population and procedures and have reported the patterns of morbidity, physical growth and dietary intake of the village children (BROWN *et al.*, in press; BROWN *et al.*, in press; BLACK *et al.*, 1982a; BLACK *et al.*, 1982b).

Of the 197 children in the longitudinal studies, a subgroup of 70 children, five to 18 months old at the beginning of the study, was selected for assessment of dietary intake. For each child, studies of dietary intake were scheduled monthly on days that the child had no serious illness. All foods consumed by the children on the days of study were weighed by a dietician, who observed the children in their homes during all their waking hours.

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The age at introduction and patterns of consumption of weaning foods have been described (BROWN *et al.*, in press). Briefly, the weaning process in these villages can be described as a gradual reduction in the amount of breast milk received, from 632 g/day in children five to 11 months to 368 g/day in children 24 to 30 months, a later reduction in the proportion of children receiving breast milk, and a concurrent gradual introduction of greater amounts and types of supplementary foods. Children were almost universally breastfed up to and beyond 24 months of age, and weaning foods were introduced relatively late and in scanty amounts. Cereals, sugar, fruits, roots, tubers and dairy products were the foods introducted earliest, and they provided most of the nutrients not received from breast milk. Cereals, most commonly rice, were sometimes prepared with sugar and cow's milk or water to form a liquid porridge especially for children; however, most weaning foods were selected from items of the adults' diets.

Meals were prepared in kitchens attached to the house and cooked on clay stoves fuelled by wood, straw or cow dung. Food was cooked and stored in clay or metal pots. To determine the ambient temperature at which foods were prepared and stored, a thermometer was left in the kitchen during the day of the dietary study and the temperature was recorded by the dietician at 6 a.m. and 12 noon and by the field worker at 6 p.m. during the day of a dietary study. Since foods were most often prepared and stored in the morning and early afternoon, the noon temperature was used in all analyses.

During monthly studies of dietary intake between May 1978 and March 1979, samples of foods or water consumed by 40 of the diet study children (in 39 households) were collected to be tested for bacterial contamination. The remaining 30 children were exclusively breastfed when these studies began. These samples, which were obtained from the child's cup or bowl just before the food was served to the child, were placed in sterile glass vials. Samples of all soft and liquid foods were saved; dry foods, however, were not collected for testing. The vials were kept in an insulated box with frozen cold packs until the end of the day. All specimens were then transported to the laboratory and processed immediately. Laboratory tests done before these studies indicated that bacterial colony counts of food or water changed very little during 12 hours of storage in a cold box.

Water and liquid specimens were mixed thoroughly and 0·1 ml of each specimen and of serial 10-fold saline dilutions (to 10^{-6}) were spread over pre-dried plates. Soft foods were first blended and serial dilutions prepared. The lowest dilution plated was the first that could be quantitatively pipetted, and the highest was 10^{-6}. Plating media included trypticase soy, MacConkey's and trypticase-tellurite-gelatin agars. For water, but not for food specimens, 0·1 ml was added to triple-strength bile peptone, enriched overnight, and plated on trypticase-tellurite-gelatin agar. After incubation at 37°C for 24 hours, the number of colonies on the plates was counted. Plates were examined for salmonellae, shigellae and vibrios by standard methods (EDWARDS & EWING, 1972). To test for enterotoxigenic *Escherichia coli* (ETEC), five lactose-positive colonies with typical *E. coli* morphology and a pool of 10 other lactose-positive colonies were removed from MacConkey's agar and stored on nutrient agar slants. These five colonies and pool of 10 colonies were tested for heat-labile toxin (LT) by the Chinese hamster ovary cell assay and for heat-stable toxin (ST) by the infant mouse assay within one month after initial isolation (MERSON *et al.*, 1976).

Previous reports have documented the incidence, aetiology, and seasonality of diarrhoea among study children (BLACK *et al.*, 1980, 1981). To evaluate the relationship between contamination of food or water and diarrhoea incidence, we analysed these variables for 33 of the 40 diet study children. These children were selected because they were present for the full year of morbidity surveillance and diet assessment and because they had had at least 10 food and 10 water specimens studied for bacterial contamination.

The seasonality of ETEC diarrhoea in study children is further analysed in relation to environmental temperature and rainfall. In addition, the seasonality of ETEC diarrhoea among residents of the entire Matlab field research area is related to concurrent climatic factors. Data on ETEC seasonal incidence are taken from a study done in the diarrhoea treatment centre serving this area (BLACK *et al.*, 1980). Information on rainfall, temperature and humidity was obtained for each month between February 1977 and January 1979 from the Meteorological Department of the Government of the Peoples' Republic of Bangladesh.

In this analysis, the presence of *E. coli* in a food or water sample was regarded as evidence of faecal contamination. This is in accordance with recommendations on the bacteriological safety of water and dietary foods, in which the recovery of any *E. coli* or faecal coliforms is considered unacceptable (STANDARD METHODS, 1976; INTERNATIONAL COMMISSION, 1974).

Results

Of 470 food specimens, 70% were cooked rice, 16% were cow's or goat's milk and 15% were other types of food, including special weaning foods made of rice or wheat flour and milk. 41% of these foods had detectable levels of *E. coli*, and all but two samples had detectable bacteria. 49% of milk specimens contained *E. coli*, compared with 37% of rice specimens ($p<0·05$). 57% of other foods contained *E. coli*, again significantly higher than rice ($p<0·01$). The frequency of contamination of foods with *E. coli* rose markedly with increasing environmental temperature ($p<0·001$) (Table I). In specimens containing *E. coli*, the average colony counts of *E. coli* in each type of food were 100-fold greater in foods collected on the hottest days than in those collected on relatively cool days.

The common practice of cooking foods in the early morning and storing them at ambient temperatures for consumption later in the day also appeared to encourage the growth of *E. coli*. Ten (30%) of 33 samples of milk consumed within one hour of boiling contained *E. coli*, compared with 27 (68%) of 40 samples of milk consumed after more than one hour of storage in the home ($p<0·005$). Furthermore, on 20 occasions, milk was prepared in the morning, some drunk immediately, and some later in the day; on 14 (70%) of these days, the *E. coli* counts were higher (usually one log or more) after storage than they had

Table I—Level of contamination with *Escherichia coli* in food consumed by weaning-age children* and relation to environmental temperature

Food Type and Noontime Indoor Temperature (°F)	Number of Specimens	Percentage of Specimens by *Escherichia coli* Colony Count per g or ml							Geometric Mean Colony Count of Specimens with *E. coli*
		0	10^0-$10^{1\dagger}$	10^2	10^3	10^4	10^5	10^6-10^7	
Rice									
<75	85	79	9	5	4	2	1	—	4×10^2
75-84·9	113	72	4	4	11	3	4	3	4×10^3
>85	130	48	4	13	15	11	5	4	6×10^3
Milk									
<75	25	64	12	24	—	—	—	—	1×10^2
75-84·9	28	57	14	7	11	4	—	7	7×10^2
>85	20	25	—	—	20	10	40	5	6×10^4
Other									
<75	14	64	14	14	—	7	—	—	3×10^2
75-84·9	24	58	8	8	—	17	8	—	4×10^3
>85	31	23	3	3	10	32	23	6	3×10^4
All									
<75	124	74	10	10	2	2	1	—	3×10^2
75-84·9	165	67	7	5	9	5	4	3	3×10^3
>85	181	41	3	10	14	14	12	4	1×10^4
Total	470	59	6	8	9	8	6	3	6×10^3

*6-30 months old †1-99 colonies

Table II—Level of contamination with *Escherichia coli* in water consumed by weaning-age children* and relationship with environmental temperature

Water Source and Noontime Indoor Temperature (°F)	Number of Specimens	Percentage of Specimens by *Escherichia coli* Colony Count per ml							Geometric Mean Colony Count of Specimens with *Escherichia coli*
		0	10^0-$10^{1\dagger}$	10^2	10^3	10^4	10^5	10^6-10^7	
Tubewell									
<75	25	68	20	12	—	—	—	—	9×10^1
75-84·9	37	59	19	8	11	3	—	—	4×10^2
>85	55	47	11	16	18	7	—	—	9×10^2
River/canal									
<75	60	50	37	10	3	—	—	—	6×10^1
75-84·9	44	50	36	5	5	2	2	—	1×10^2
>85	15	47	13	13	20	—	7	—	1×10^3
Surface/Tank									
<75	16	31	44	19	6	—	—	—	7×10^1
75-84·9	85	58	11	8	18	5	—	1	1×10^3
>85	138	42	4	16	26	9	2	1	2×10^3
All									
<75	101	51	34	12	3	—	—	—	7×10^1
75-84·9	166	56	19	7	13	4	1	1	4×10^2
>85	208	44	6	16	24	8	2	1	2×10^3
Total	475	50	17	12	15	5	1	1	6×10^2

*6-30 months old †1-99 *Escherichia coli* colonies

been shortly after preparation. A similar relationship between storage time and *E. coli* counts was noted for other foods, particularly for the foods prepared specifically for infants using rice powder, sugar and milk or water. Rice was usually eaten shortly after boiling and not after prolonged storage; however, when rice was stored *E. coli* counts were often quite high.

Of 475 drinking water specimens, 50% contained *E. coli*, and all but three contained bacteria (Table II). The frequency of contamination of water with *E. coli* also increased with environmental temperature ($p<0{\cdot}001$); however, in each temperature category, the number of *E. coli* in water specimens was 10-fold lower than in food specimens. Water from the various available sources had similar levels of contamination;

water from wells was as frequently and heavily contaminated (and demonstrated the same relationship with temperature) as surface water (Table II).

The 33 children studied had diarrhoea incidences in the year of study ranging from zero to 14 episodes. They had incidences of ETEC diarrhoea ranging from zero to five episodes, of *Shigella* diarrhoea from zero to three episodes, and of rotavirus diarrhoea from zero to two episodes. To categorize the level of exposure to faecal contamination for children, we used the proportion of food or water specimens that contained detectable *E. coli*. The proportion of food samples with *E. coli* ranged from zero to 83% and of water samples from 12 to 84%. We found that the proportion of food containing *E. coli* was positively correlated with children's annual incidence of ETEC diarrhoea ($r=\cdot35$, $p<0\cdot05$). The proportion of foods with *E. coli* contamination was not significantly correlated with the incidence of rotavirus, *Shigella* or all diarrhoea. The proportion of contaminated drinking water specimens was not correlated with any specific type of diarrhoea or with all types of diarrhoea. ETEC producing both ST and LT were found in two of 65 foods specimens examined and in none of 70 water specimens. Diarrhoea associated with ST/LT *E. coli* developed in one of the two children who ate food containing ETEC.

Non 0 group 1 vibrios were found in 92 (19%) of 475 water specimens and in none of the food specimens. It was not possible to relate any episodes of diarrhoea associated with non 0 group 1 vibrios to drinking water containing these organisms. One asymptomatic infection with a non 0 group 1 *Vibrio* was detected in a child who had drunk water containing those vibrios on the previous day. No salmonellae or shigellae were found in food or water.

Diarrhoea associated with ETEC occurred seasonally in children enrolled in this longitudinal study. The highest monthly incidence of ETEC diarrhoea occurred from April to August, which had high average temperatures and heavy rainfall (especially in May and June).

The seasonality of ETEC diarrhoea was also evaluated among patients at the central diarrhoea treatment centre that provides services for the 280,000 persons living in the field research area. In a two-year study, ETEC diarrhoea was found to have a marked seasonal distribution (BLACK *et al.*, 1980). This seasonal occurrence was strikingly associated with the average monthly environmental temperature (Fig. 1). The seasonality did not correspond with the pattern of heavy rainfall.

Discussion

In lesser developed countries, exclusively breastfed children of undernourished mothers will usually need supplementary foods after they reach three to six months of age to maintain optimal growth (BROWN *et al.*, in press; BROWN *et al.*, in press; WATERLOW & THOMSON, 1979). In this study, much of the food and water given to weaning-age children had faecal contamination, as indicated by the frequent recovery

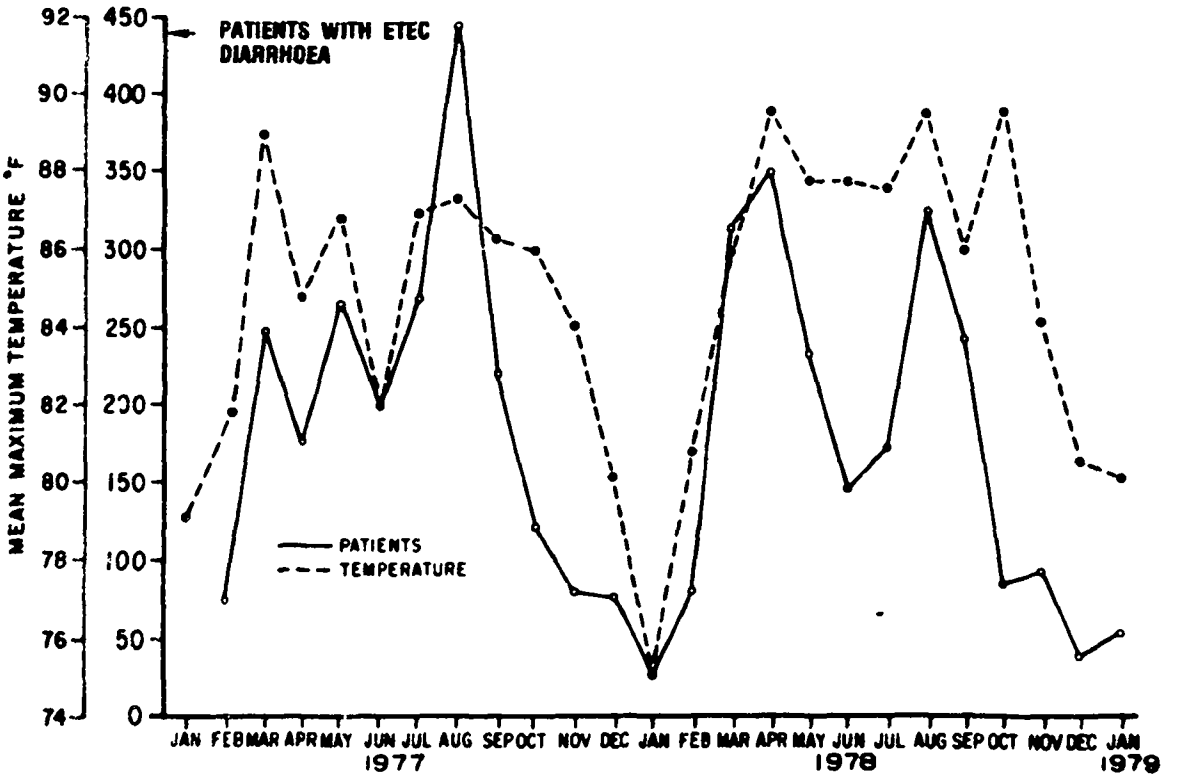

Fig. 1. Number of patients with enterotoxigenic *Escherichia coli* (ETEC) diarrhoea at the Matlab Treatment Center and ambient temperature February 1977-January 1979.

of *E. coli* in high colony counts from these specimens. Consumption of such food and water is likely to increase the risk of acquisition of enteropathogens normally spread by the faecal-oral route.

Although specimens of water more frequently contained *E. coli* than did specimens of food, the number of *E. coli* in contaminated foods was generally ten times higher than that in contaminated water. There are many possible reasons for these higher levels of contamination. Raw ingredients and water used to prepare foods are often faecally contaminated, and inadequate cooking permits survival of bacteria (BARRELL & ROWLAND, 1979). Containers and utensils used to prepare food may still be heavily contaminated, even when washed (ROWLAND *et al.*, 1978). Food stored in the house can be contaminated by household members, animals or insects. Perhaps the most critical factors determining the number of organisms, such as *E. coli*, found in foods are the duration and conditions of storage (BARRELL & ROWLAND, 1979). Many bacteria would be expected to multiply in foods kept at the 80 to 90°F temperature commonly found in homes in many developing countries in the summer months (INGRAHAM, 1962). This temperature-related bacterial proliferation, combined with more frequent faecal contamination of cooking water, probably accounts for the 100-fold higher levels of *E. coli* in foods during the hotter months. Such proliferation of *E. coli* may be particularly important, because enterotoxigenic *E. coli* appear to require a relatively high inoculum to cause diarrhoea (DUPONT *et al.*, 1971).

The importance of bacterial contamination of weaning foods was demonstrated in this study by the observation that the proportion of a child's food samples that contained *E. coli* was significantly related to that child's incidence of diarrhoea associated with ETEC, the most frequent enteropathogens causing diarrhoea among the study children (BLACK *et al.*, 1982a). This finding and our previous studies suggest that foods are important vehicles in the transmission of enterotoxigenic *E. coli* (BLACK *et al.*, 1982b). Furthermore, the seasonal variation in the level of contamination of foods is the most likely explanation for the seasonality of enterotoxigenic *E. coli* diarrhoea and its striking association with environmental temperatures. Seasonal differences in the survival of enteropathogens in faecally polluted water may also play a role in determining the frequency and degree of contamination of food and of drinking water.

Recognizing the need for adequate amounts of nutritious weaning foods for infants, a recent WHO/UNICEF meeting on infant feeding recommended that "foods locally available in the home can be made suitable for weaning and their use should be strongly emphasized" (World Health Organization, 1973; WHO/UNICEF Meeting, 1979). At the same time, we must concern ourselves with the hazard of faecal contamination of such foods and strive for foods that can be prepared hygienically and eaten immediately or stored safely until consumption. Only in this way can the dual purposes of reducing malnutrition and diarrhoea morbidity be achieved.

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