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Bacterial contamination of stored water and stored food: a potential source of diarrhoeal disease in West Africa

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SUMMARY

The food and water hygiene in two Liberian communities was studied in a house-to-house diarrhoea survey. The level of contamination with enterobacteria of drinking water stored in the households was significantly higher than at the water sources. Food hygiene standards were low, particularly in the urban slum where storage of cooked food for long periods led to bacterial multiplication at high levels. Infant foods were particularly heavily contaminated. It is concluded that when water supply programmes are planned, the presence of other risk factors for water-related diseases should be investigated. To ensure maximum health benefits, water projects should as a rule be accompanied by other interventions.

INTRODUCTION

Diarrhoeal diseases are considered the most important group of water- and excreta-related infections, and are a leading cause of child mortality and morbidity in developing countries (Snyder & Merson, 1982). An adequate quantity of safe drinking water and facilities for the proper handling and disposal of human waste are essential for the prevention of diarrhoeal diseases. Consequently the supply of safe water and basic sanitation were included in the concept of comprehensive primary health care at the World Health Conference at Alma Ata in 1978, and 1980–1990 has been declared the International Drinking Water Supply and Sanitation Decade. The predicted improvement in health status measured by a decline in child morbidity and mortality rates was initially based more on historical experiences and common sense than on epidemiological results. By reviewing 67 studies from 28 countries (Esrey, Feachem & Highes, 1985) it was shown that a substantial reduction in diarrhoea morbidity and mortality can be expected from well-conducted water supply and sanitation programmes, although there is a considerable variation in the recorded impact of these projects. The magnitude of the impact depends greatly on the presence of other risk factors. More knowledge of interactions with these factors will enable the appropriate type

of intervention to be targeted, and will point to simultaneous activities necessary to carry out.

Some risk factors of possible importance were studied in a house-to-house diarrhoea survey in Liberia, West Africa (Højlyng *et al.* 1985; Højlyng, Mølbak & Jepsen, 1986; Mølbak, Højlyng & Gaarslev, 1988). Particular attention was paid to water and food hygiene. The practices of a population residing in an urban slum area and a typical rural subsistence farming area were studied, and the degree of bacterial contamination of source water, stored water and prepared food was investigated.

MATERIALS AND METHODS

Study population. Two populations, an urban slum and three rural villages, were selected for the study.

The urban slum, West Point, is a shanty town situated on a 0·25 km^2 peninsula extending from the capital city Monrovia into the Atlantic Ocean. The estimated population was at the time of the survey 30000 living under very poor conditions primarily in one-storey tin shacks. Piped water was supplied to 40–50 standpipes through a surface pipeline. These outlets were unable to meet the demands of the population, and an abundance of illegal connections had been made. Since non-return valves were never installed, the risk of contamination of the pipes was substantial at low-pressure leakage points. Facilities for waste disposal were almost non-existent. The population was forced to use either the beach or temporary constructions on the beach line for excreta disposal. Because of the special composition of the ground, holes for latrines or septic tanks were difficult to dig. Flush toilets in the houses were seen on few occasions, and three community toilets were under construction, but not yet functional. No system for removal of waste was working. Waste was dumped primarily in the ocean or in a lagoon. An open sewage drain running from Monrovia city to the ocean and passing through West Point was stagnant due to blocking garbage.

The rural district, Bong County, is situated 200 km inland in a forest area. Three villages were selected, in which a total population of approximately 3500 were living in adobe houses with almost no facilities. Public hand pumps were installed in two of the villages, but they were either 'privatized' or in poor operational shape, and were insufficient as village supply. The main water supply came from hand-dug wells, rivers and creeks. The majority of the population used the forest to dump human and household waste. Only one third used latrines for excreta disposal, and these latrines were often in poor condition. Streets and yards were as rule swept and fairly clean.

The study was carried out during the dry and late dry season, January–April 1983. Households with children aged 6–59 months were asked to participate in the study. A household was defined as an extended family living together and sharing a cooking pot. Households were visited repeatedly until all children living more than 4 weeks in the household were seen. The refusal and fall-out rates were low in both areas, less than 4%. Information was recorded on the socio-economic condition, education, housing, family size, ethnic group, drinking water supply, storage of water, storage of prepared food, and personal and domestic hygiene.

Group A comprised 144 households from West Point, randomly sampled from a house-to-house map.

Group B consisted of 41 households with children attending a public health clinic at West Point, complaining of diarrhoea.

Although these two groups were selected differently, we did not observe any differences in food and water practices, and the results from the bacteriological examinations were also identical. Consequently the results from these two groups are not reported separately in the present paper.

Group C included all households with children in the age group 6–59 months from the three villages in Bong County. A total of 260 households was visited.

Collection of water and food samples. One water specimen from source water (e.g. standpipe, handpump, well, creek), and one specimen from stored water (e.g. pot, tub) was collected in each household. When available, one or two samples of cooked food were collected, and type of food and time since preparation were recorded. For ethical and cultural reasons, only small quantities of food were collected.

All specimens were sampled in sterile containers, and kept in an insulated box with ice packs (5–10 °C) until processing within 12 h.

Culturing for enterobacteria. Approximately 0·2 g of food was prior to culturing taken by a sterile spoon, weighed, and suspended in 2 ml of sterile phosphate buffered saline (PBS, pH 7·2). Each food suspension, and the undiluted water samples, were seeded on three plates (SSI medium, Statens Seruminstitut, Copenhagen, Denmark; a medium with low selectivity giving growth to enterobacteria and vibrios), in quantities of 0·01, 0·1 and 1·0 ml, and incubated for 24 h at 35 °C. The SSI medium has a high differentiating capacity, and evaluation of individual cultures for lactose fermentation, H_2S production and the phenylalanine deaminase reaction is straightforward. On the basis of these three reactions and in addition the size and the morphology of individual colonies, the different types of colonies, from the plate with the best differentiation of individual colonies, were counted separately. The colonies were then preserved in beef extract agar for further identification. When the isolated strains were re-cultured, they were examined for β-galactosidase by the ONPG test and β-glucuronidase by the PGUA (4-nitrophenyl-β-D-glucopyranosiduranic acid) test, oxidase reaction, and ability to grow in the depth of a semi-solid motility medium containing glucose (Kilian & Bülow, 1976; Lautrop *et al.* 1979). 'Enterobacteria' were defined as ONPG and/or PGUA positive, oxidase negative facultative anaerobic bacteria. The number of bacteria were reported as colony forming units (c.f.u.)/100 g material. The sensitivity of this method is 10^2 c.f.u./100 g (water and undiluted, liquid food), and 10^3 c.f.u./100 g for food diluted in PBS.

RESULTS

Food and water practices. Drinking water was stored in most households in both areas. Table 1 shows that many families stored the water for a long time in large, open containers. Twenty-nine families from the urban slum kept their drinking

Table 1. *Type of water storage*

	West Point		Bong County	
	No. of households	(%)	No. of households	(%)
No storage	9	5	1	0
Storage in:				
Open container	45	24	158	61
Closed container	96	52	88	34
Refrigerator	29	16	0	0
Ceramic filter	0	0	1	0
No information	6	3	12	5
Total	185		260	

Table 2. *Storage of prepared (cooked) food*

	West Point		Bong County	
	No. of households	(%)	No. of households	(%)
No storage	34	18	151	58
Usually storing	136	74	73	28
Sometimes storing	12	6	31	12
No information	3	2	5	2
Total	185		260	

water in a refrigerator. However, this water was rarely collected directly from a water standpipe, but often from an unprotected container in the house.

Table 2 shows that storage of prepared, cooked food was very common at West Point, where 81 % of the households usually or sometimes stored food. The proportion of households from the rural area storing food was significantly lower, 41 % ($P < 0{\cdot}0001$, χ^2). Food was usually stored on a table or on the floor in a bowl which was sometimes wrapped in a piece of cloth. The ambient temperature was very high, often 30–36 °C. The members of the household, especially the children, could have several meals per day from such a bowl with rice and sauce.

The mean storage time of the samples collected at West Point was longer, 7 h, range 0–24 h, compared to a mean storage time of 4 h, range 0–17 hours, in the rural area.

Bacteriological examination of water. The water from 27 taps at West Point was analysed. Twenty-four of these taps were public, and three were private. As shown in Table 3, five (19 %) of the taps were contaminated in the range 10^2–10^4 c.f.u./100 ml, while the samples collected from the remaining taps, including all the private taps, showed no growth of enterobacteria. One hundred and fifty-three samples of stored water were analysed. Seventy-one (46 %) had colony counts ranging from 10^2–10^7 c.f.u./100 ml, Table 3. Storage of water influenced significantly the proportion of samples contaminated ($P < 0{\cdot}025$, χ^2).

Water from 20 water sources in the rural area was analysed. The water was

Table 3. *Enterobacteria in drinking water, West Point*

c.f.u.*/100 ml	Source water		Stored water	
	No. of samples	(%)	No. of samples	(%)
$<10^2$	22	81	82	54
10^2–10^3	1	4	11	7
10^3–10^4	4	15	28	18
10^4–10^5	0	0	21	14
10^5–10^6	0	0	10	7
10^6–10^7	0	0	1	1
Total	27		153	

* Colony forming units.

Table 4. *Enterobacteria in drinking water, Bong County*

c.f.u.*/100 ml	Source water		Stored water	
	No. of samples	(%)	No. of samples	(%)
$<10^2$	3	15	6	4
10^2–10^3	1	5	9	6
10^3–10^4	5	25	29	21
10^4–10^5	10	50	66	47
10^5–10^6	1	5	27	19
10^6–10^7	0	0	2	1
Total	20		139	

* Colony forming units.

taken from 4 hand-pump stands, 13 shallow wells, 2 waterholes and 1 semi-dry river, all of which were used for drinking water supply. Table 4 shows that only water from three hand-pumps showed no growth of enterobacteria, the rest of the samples had colony counts in the range 10^2–10^6 c.f.u./100 ml. Samples of stored water were collected in 139 households. One hundred and thirty-three samples (96%) were contaminated. Although a larger proportion of the samples of stored water was contaminated as compared to the supplies, the difference was not statistically significant.

Bacteriological examination of food samples. A total of 218 food samples was analysed, 108 samples from West Point (16 samples of supplementary food and breast-milk substitutes, and 92 samples of adult food, mostly rice and sauce), and 110 samples from Bong (adult food), Table 5.

Sixty-three of the food samples (68%) collected at West Point and 72 samples (65%) from Bong were contaminated. The difference between the two areas was not significant, however; the proportion of heavily contaminated samples (i.e. $> 10^4$ c.f.u./100 g) was significantly higher at West Point (63% versus 39% in the rural area, $P < 0{\cdot}005$, χ^2).

Sixteen samples of babyfood were examined. These were all collected at West Point, and had been stored up to 8 h. Eight of the samples were

Table 5. *Enterobacteria in prepared food*

	West Point				Bong County	
	Infant food		Adult food		Adult food	
c.f.u.*/100 ml	No. of samples	(%)	No. of samples	(%)	No. of samples	(%)
$< 10^3$	3	19	29	32	38	35
10^3–10^4	0	—	5	5	29	26
10^4–10^5	4	25	29	32	22	20
10^5–10^6	1	6	14	15	13	12
10^6–10^7	3	19	7	8	7	6
10^7–10^8	5	31	8	9	1	1
Total	16		92		110	

* Colony forming units.

formulas ('bottlemilk'), seven commercial baby cereals, and one traditional 'ricewater'. Thirteen (81 %) of the samples were contaminated, all in the range 10^4–10^8 c.f.u./100 g.

DISCUSSION

This paper presents results from a survey on the aetiology of childhood diarrhoea in Liberia. Results from the child and stool examinations have been reported (Højlyng *et al.* 1985; Højlyng, Mølbak & Jepsen, 1986; Mølbak, Højlyng & Gaarslev, 1988). As a part of the survey, a number of possible diarrhoea risk factors were studied.

The water supply system at West Point was badly maintained, and was hooked up with illegal connexions. Nevertheless, the water provided was of a fairly acceptable standard, and much better than the water from the rural area (19 % versus 85 % of the samples contaminated, $P < 0{\cdot}0001$, Fisher's exact test). However, the diarrhoea prevalence was higher at West Point (group A): 80/237 (33·8 %), compared to 108/488 (22·1 %) in the rural area, group C ($P < 0{\cdot}005$, χ^2).

This emphasizes that morbidity from diarrhoeal diseases in a poor community such as West Point is not only dependent on the quality of the water supply. Detectable health benefits from improving water supplies should not be expected as long as other major risk factors are present and important routes of transmitting diseases are not interrupted. This has been discussed in detail earlier, and theories for the explanation have evolved (Shuval *et al.* 1981; Esrey, Feachem & Hughes, 1985).

In Bong County water from only three handpumps was of an acceptable standard. The great majority of the water sources were contaminated with enterobacteria. The problem was aggravated since very few of the households had access to the water pumps, and other pumps were in poor operational condition as no regular maintenance was carried out.

An important problem in both areas was storage of water in the households. The extent of contamination of bacteria of possible animal and human faecal origin

was at West Point influenced significantly by storage. Storage of large quantities of drinking water in the households is common in most developing countries. This practice will not be changed unless water is provided accessible at a short walking distance, and water supply programmes are accompanied by health education promoting hygienic handling of water. It is striking that the six household samples (4%) without growth of enterobacteria collected in the rural area came from families using water from uncontaminated hand-pumps, and from one household provided with a ceramic water filter.

Unhygienic handling of food was a problem in both areas, most serious in the urban slum. It was a common practice to prepare food far in advance of consumption, and leave it at ambient temperature for up to 24 h. Stored food was very often consumed cold, or reheated for a short period. Under such conditions pathogenic bacteria will persist and multiply in food to potentially dangerous levels. Two-thirds of the samples examined in the present survey were contaminated, and the colony counts were significantly higher at West Point than at Bong County, probably as a result of a longer storage time and higher faecal transmission rates in the urban slum. There are several explanations why food storage was more common at West Point. Charcoal was expensive in the urban area, whereas firewood could be collected in the rural district. More broken families were seen at West Point, and many women worked long distances away from their homes. These women were not able to prepare food more than once a day.

The standard of water at West Point was quite good, and even water stored in the households was of a significantly better standard than the water from the rural area ($P < 0{\cdot}0001$, χ^2). But microbiological quality of the food from West Point was very poor. This suggests that water quality has no major influence on the standard of the food in a community where faecal transmission is high and prolonged food storage is a common practice. The results from the rural area were different: 96% of the household water samples were contaminated, whereas 'only' 66% of the food samples were contaminated. It is thus tempting to suggest that the water quality is a more critical factor in an area like Bong, where the general hygiene was better than in the crowded and contaminated urban slum. Such a community is more likely to obtain health benefits from a well-conducted water supply and sanitation programme (Shuval *et al.* 1981).

Infancy and the weaning period is a particularly vulnerable period of life with a high morbidity and mortality from diarrhoeal diseases. In the present study, the prevalence of diarrhoea was very high in the urban slum, where the proportions of children who were breast fed were smaller than those in the rural area. The mean duration of breastfeeding was also found to be shorter, 12 months compared to 18 months, and supplementary food was introduced at an earlier age. Breast-milk substitutes and commercial baby cereals, believed to be fashionable, western and modern were increasingly used at West Point, where 35% of the children were or had been bottle-fed compared to only 7% of the rural children. Although the number of infant foods examined is small, the appalling contamination emphasizes the danger of bottle-feeding and the danger of unhygienic practices during the period of weaning. In addition, cryptosporidiosis was, in the present study, found

to be associated with bottle-feeding (Højlyng, Mølbak & Jepsen, 1986), and campylobacter infection was associated with supplementary feeding (Mølbak, Højlyng & Gaarslev, 1988).

When water supply and sanitation projects are initiated, hygienic practices and other risk-factors for water-related diseases should be analysed carefully in detail. Such investigations will point to other activities needed in the community to ensure significant health benefits. It is of paramount importance that water and sanitation projects should be accompanied by health education programmes and the promotion of personal and domestic hygiene. The hazardous practices of storing large quantities of drinking water in open containers as well as storage of cooked food must be discouraged as must the potential dangers of bottle-feeding and unhygienic practices during weaning. Breastfeeding and hygienic handling of food should be encouraged.

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