The Central African Journal of Medicine

Supplementary Issue to 1992 Volume 38,
1991 University of Zimbabwe Annual Research Day

CENTRAL AFRICAN
JOURNAL OF MEDICINE

Vol. 38, No. 4, April, 1992

Bacterial contamination of food and household stored drinking water in a farmworker community in Zimbabwe

C SIMANGO,* J DINDIWE, G RUKURE

SUMMARY

Food and water samples, collected from the homes of farmworkers with children less than five years old, were cultured for *Escherichia coli* (which was used as an indicator of faecal contamination) and bacterial enteric pathogens. Sixteen percent of the food samples and 41 pc of the household stored water

Blair Research Laboratory
Ministry of Health
PO Box 8105
Causeway, Zimbabwe
**Current address and correspondence:*
Department of Medical Laboratory Technology
University of Zimbabwe Medical School
PO Box A 178
Avondale, Zimbabwe

Vol. 38, No. 4, April, 1992

samples had *E.coli*. Ten percent of the foods had high *E.coli* counts greater than 10^4 counts per ml or g of food. Most of the foods were stored for more than 12 hours. Bacterial enteric pathogens were isolated in low percentages in foods and water except *Aeromonas* which was very common in household stored water.

The majority of the faecally contaminated water samples had low *E.coli* counts (less than 20 *E.coli*/100 ml) and 61 pc of the water was stored for less than 12 hours. There was no relationship between faecal contamination of the water and period of storage in the home. Household stored water had a higher percentage of samples contaminated with *E.coli* than the tap water which was used to fill the storage vessels. Household stored drinking water is a major route of transmission of *Aeromonas* in the rural community which was studied.

INTRODUCTION

Diarrhoeal disease is one of the major problems affecting young children in the tropics. Standards of personal hygiene and public sanitation are low in many communities in developing countries and contamination of foods and drinking water with pathogenic micro-organisms may be an important source of infectious diarrhoea.

Not much work has been conducted on the role of contaminated foods and household stored drinking water in the transmission of childhood diarrhoea in developing countries. In a study which was carried out in Gambia,[1] it was observed that a very high proportion of food consumed by infants and young children was overgrown with bacteria to a hazardous degree. In another study which was carried out in Bangladesh, *Escherichia coli* was observed in 41 pc of samples of food fed to weaning age children.[2] In Zimbabwe, infants are introduced to weaning foods consisting of porridge and adult-type foods such as *sadza* (thick maize meal porridge), vegetables, meat and bread from the age of three months.

This study was carried out to assess the faecal contamination of foods fed to infants and young children and household stored drinking water as well as to detect bacterial enteric pathogens.

MATERIALS AND METHODS

The study was carried out in the Mazowe commercial farming area, 30 kilometres north of Harare. The food samples were collected from the cooked or prepared food that was available in the homes of farmworkers with children less than five years old. Each food sample was taken from the cooking utensil or storage container in the home using a sterile stainless steel spoon and was placed in a sterile wide-mouth bottle with a screw cap. The bottles with the food samples were kept in an insulated box with cold packs, maintained at 8°–10°C, and were cultured within six hours after collection.

Water samples were taken from the storage containers in the homes of the farmworkers using sterile aluminium mugs and transferred to sterile bottles with screw caps. Water samples were also collected from the public standpipes, where the farmworkers obtained their water, following the recommended water sampling procedures.[3] Information on the storage practices for food and water was obtained through interviewing the mothers or childminders and through observation.

The food samples were cultured for the detection and enumeration of *E.coli*, which was used as an indicator of faecal contamination. The *E.coli* isolates were then tested for the enteropathogenic, enterotoxigenic and enterohaemorrhagic *E.coli* strains. The food samples were also cultured for *Shigella, Salmonella, Aeromonas, Campylobacter* and *Yersinia enterocolitica*.

The solid food samples were homogenised by adding 20,0g of food to 180 ml of sterile 0,1 pc peptone water in a sterile stainless steel cup and the contents were mixed thoroughly, using a sterile food blender, before culturing. *E.coli* and its pathogenic strains were isolated and identified by preparing ten-fold serial dilutions of the food in sterile 0,1 pc peptone water and plating out 0,1 ml of each dilution in duplicate onto MacConkey agar plates. The plates were incubated at 37°C for 18–24 hours. All the lactose fermenting colonies with typical *E.coli* morphology were counted as *E.coli*. Representative colonies were confirmed as *E.coli* on the API 20E multitest system (API System, France). At least 10 *E.coli* colonies from each sample were pooled and

tested for enteropathogenic, enterotoxigenic (LT) and enterohaemorrhagic *E.coli*.

To identify enteropathogenic *E.coli*, the pooled *E.coli* colonies were serotyped with enteropathogenic *E.coli* agglutinating sera (Wellcome Diagnostics), following the manufacturer's instructions. The pooled *E.coli* colonies from each sample were also tested for the production of the heat labile toxin (LT) using the Biken test method.[4] The tests were carried out using the Biken test kits (Virion Diagnostic Laboratories, Switzerland), following the manufacturer's instructions. For the detection of enterohaemorrhagic *E.coli* 0157:H7, the pooled *E.coli* colonies were streaked on Sorbitol-MacConkey agar medium (Difco Laboratories) and the sorbitol negative colonies which grew on the medium were serotyped with 0157 and H7 agglutinating sera.[5]

For the other bacterial enteric pathogens, 20 ml of the homogenised food samples were enriched in 20 ml of double strength appropriate enrichment media before being subcultured for isolation and identification. Preston broth was used for the enrichment of *Campylobacter*. The inoculated broth was incubated at 42°–43°C for 48 hours micro-aerophilically before being subcultured onto Preston agar medium. The plates were incubated for a further 48 hours at 42°–43°C under micro-aerophilic conditions. Selenite broth was used for the enrichment of *Salmonella* and *Shigella*. The inoculated Selenite broth was incubated at 37°C for 24 hours before being subcultured onto Desoxycholate Citrate agar.

The enrichment medium for *Aeromonas* was made up of Nutrient broth supplemented with lysed sheep blood and ampicillin (20μg/ml). The broth cultures were incubated at room temperature for 24 hours before being subcultured onto the agar medium of the same composition. For the enrichment of *Yersinia enterocolitica*, Nutrient broth supplemented with lysed sheep blood and *Yersinia* selective supplement (SR 109, OXOID) was used. The inoculated broth medium was also incubated at room temperature for 24 hours before being subcultured onto *Yersinia* Selective agar medium (OXOID).

Water samples from household stored water were cultured for the same bacterial enteric pathogens as from the food samples. The same enrichment and culturing procedures used in the food samples were followed. *E.coli* was detected and enumerated using the membrane filtration method[6] and cultured on lauryl sulphate broth, supplemented with aniline blue.[7] After subculturing the enrichment broth cultures onto agar media and incubation of the agar plates, standard methods were used to identify the bacterial enteric pathogens.[8]

RESULTS

A total of 208 food samples and 216 household stored water samples were collected and cultured for *E.coli* and bacterial enteric pathogens. A further 43 tap water samples were collected from the standpipes and cultured for *E.coli* only. Sixteen percent of the food samples collected from the homes of the farmworkers had *E.coli* indicating faecal contamination (Table I). The various foods examined showed a range of 0 pc to 23 pc of samples contaminated with *E.coli*. Ten percent of the food samples had high *E.coli* counts greater than 10^4 counts per ml or g of food.

The commonest cooked or prepared foods found in the homes of farmworkers were vegetables, *mahewu* (a non-alcoholic fermented cereal gruel) and porridge. Most of the porridge was stored for less than 12 hours and had a lower percentage of samples contaminated with *E.coli* than with vegetables and *mahewu* which were stored for more than 12 hours (Table II).

The bacterial enteric pathogens were isolated in low percentages in foods and water except *Aeromonas* which was isolated in a higher percentage in stored water (Table III). Enteropathogenic *E.coli* was isolated in a higher percentage from the foods than from stored drinking water. Enterotoxigenic *E.coli* and *Campylobacter* were only isolated from stored drinking water. *Aeromonas* was isolated more often from drinking water than from foods. No *Y.enterocolitica*, *Shigella* or *Salmonella* were isolated from the various foods and stored drinking water.

Forty-one percent of the water samples had *E.coli* but the majority of the water samples had low *E.coli* counts (less than 20 *E.coli*/100 ml).

Sixty-one percent of the stored water was stored for less than 12 hours and there was no significant difference in the number of water samples contaminated with *E.coli* in relation to length of storage (Table IV).

Table I: Contamination with E.coli *in foods consumed by young children*

Type of food	Number of samples with *E.coli* counts per ml or g of food					Total number	pc
	0	10^0-10^1	10^2-10^3	10^4-10^5	10^6-10^7		
Vegetables	34	0	5	4	1	43	23
*Mahewu**	31	0	2	4	1	38	18
Porridge	32	0	1	0	2	35	9
*Sadza***	18	0	0	1	2	21	14
Meat	15	0	1	0	0	16	6
Pumpkins	11	1	2	0	0	14	21
Bread	10	0	0	0	0	10	0
Others	24	0	2	2	3	31	23
Total	175	1	13	11	9	208	16

**a non-alcoholic fermented cereal gruel*
***thick maize meal porridge*

Table II: Contamination with E.coli *in porridge, vegetables and* mahewu *in relation to length of storage*

Length of storage (hrs)	Number of samples with *E.coli* counts per ml or g of food					Total number
	0	10^0-10^1	10^2-10^3	10^4-10^5	10^6-10^7	
a) *Porridge*						
0-12	27	0	1	0	2	30
more than 12	4	0	0	0	1	5
b) *Vegetables*						
0-12	18	0	1	0	0	19
more than 12	16	0	4	3	1	24
c) *Mahewu*						
0-12	1	0	0	1	1	3
more than 12	30	0	3	2	1	36

Table III: Enteric pathogens isolated from various foods and household stored drinking water

Enteric pathogens isolated	Foods (n = 208)	Water (n = 220)
Enteropathogenic *E.coli*	1,9 pc	0,9 pc
Enterotoxigenic *E.coli* (LT)	0 pc	0,9 pc
Campylobacter	0 pc	1,4 pc
Aeromonas	1,4 pc	17,3 pc

Table IV: Contamination with E.coli *of stored water in relation to length of storage*

Period of storage (hrs)	Percentage of samples with *E.coli* counts per 100 ml					Total number	Total pc of samples with *E.coli*
	0	1-20	21-50	51-100	over 100		
0-12	60	12	5	5	19	131	41
Over 12	55	9	7	8	20	85	45

Table V: Contamination with E.coli *of stored water in relation to whether the storage vessel was covered or not*

	Percentage of samples with *E.coli* counts per 100 ml						
	0	1-20	21-50	51-100	over 100	Total number	Total pc of samples with *E.coli*
Storage vessel covered	59	11	6	5	19	113	41
Storage vessel not covered	57	10	7	6	20	101	43

Table VI: Contamination with E.coli *of stored water in relation to whether some of the water had been used or not*

	Percentage of samples with *E.coli* counts per 100 ml						
	0	1-20	21-50	51-100	over 100	Total number	Total pc of samples with *E.coli*
Some water in the storage vessel used	57	9	7	5	22	169	43
Water in the storage vessel not used	66	13	4	6	11	47	34

Table VII: Contamination with E.coli *of stored tap water and direct tap water*

	Percentage of samples with *E.coli* counts per 100 ml						
Source of water	0	1-20	21-50	51-100	over 100	Total number	Total pc of samples with *E.coli*
Stored tap water	59	10	7	7	19	216	41
Direct tap water	72	23	0	0	5	43	28

Ninety-nine percent of the families used the water storage vessels to collect water and all three water samples which were taken from the storage vessels which were not used to collect water had *E.coli*. Approximately one half of the water storage vessels were covered (Table V) and there was no significant difference in contamination with *E.coli* of the stored water whether the storage vessel was covered or not ($X^2 = 0,089$; $P>0,5$. A higher percentage of household stored water in the storage vessels where some of the water had been used had E.*coli* than stored water which had not been used as shown in Table VI.

Household stored tap water had a higher percentage of samples contaminated with *E.coli* than tap water which was used to fill the storage vessels (Table VII) but the difference was not significant ($X^2 = 1,329$; $P>0,1$). However, there was a significant difference when a comparison was made between heavily contaminated samples (over 100 *E.coli* counts/100 ml) and the rest ($X^2 = 6,158$; $P<0,02$).

DISCUSSION

The results of the present study show that much of the food and water consumed by the community had faecal contamination, as indicated by the frequent recovery of *E.coli*.

Consumption of such food and water is likely to increase the risk of acquisition of enteropathogens normally spread by the faecal-oral route. Although a large percentage of household stored drinking water had *E.coli*, most of the contaminated water had low *E.coli* counts (less than 20 *E.coli*/100 ml).

The low *E.coli* counts in the household stored water could be due to the fact that most of the families used the same containers for water collection and storage. The use of separate containers for water collection and storage has been shown to be a factor causing the gross contamination of stored water[9] since the storage vessels not used for collecting water may not be cleaned as often as those which are used for both purposes.

There was evidence of faecal contamination of water during storage in the home since a higher percentage of household stored water had *E.coli* than tap water which was used to fill the storage vessels. Also, a higher percentage of household stored water in the storage vessels where some of the water had been used had *E.coli* than stored water which had not been used.

Similar observations were noted in Egypt[10] where *E.coli* and faecal streptococci were higher in zir (conical-bottomed earthenware vessel) water than in tap water which was used to fill the zir and in Malawi[9] where a considerable contamination of water with faecal coliforms during household storage was observed.

The number of *E.coli* in contaminated foods was generally much higher than in contaminated water and a high percentage of the faecally contaminated foods had high *E.coli* counts (more than 10^4 *E.coli* counts/ml or g of food). The high *E.coli* counts in the stored foods may indicate unhygienic handling and storage of food which resulted in the contamination of food with *E.coli* and the multiplication of the bacterium in the food.

Specific bacterial enteric pathogens were isolated in low percentages in food and water except *Aeromonas*, which was isolated frequently in water and this may be due to the fact that *Aeromonas* is an aquatic organism. Stored drinking water had more bacterial enteric pathogens than stored food. Although food and water are likely sources of enteric pathogens, low isolation rates of these organisms in endemic areas, as noted in the present study, have been observed by other workers as well.

For example, low isolation rates of bacterial enteric pathogens were observed in foods and stored water collected from the homes of people in rural areas in Thailand.[11, 12] In the present study, stored cooked or prepared food and drinking water were observed to be potential sources of enteric pathogens in the community which was studied since high percentages of stored food and drinking water were faecally contaminated and some had bacterial enteric pathogens.

Stored drinking water appears to be the main source of *Aeromonas*, an organism which has been associated with diarrhoea. Health education in personal and domestic hygiene is required in this community in order to reduce the transmission of enteric pathogens through contaminated stored food and drinking water.

ACKNOWLEDGEMENTS

We thank the Secretary for health for permission to publish. We are grateful to Martin Ndhlovu and Christopher Nhandara for technical assistance.

REFERENCES

1. Barrel R A E, Rowland, M G M: Infant foods as a potential source of diarrhoeal illness in rural West Africa, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1979; 73: 85–90.
2. Black R E, Brown K H, Becker S, Abdul Alim A R M, Merson M H: Contamination of weaning foods and transmission of enterotoxigenic *Escherichia coli* diarrhoea in children in rural Bangladesh, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1982; 76: 259–264.
3. WHO: *Guidelines for Drinking Water Quality*, Health criteria and other supporting information, World Health Organisation, Geneva, 1984; II.
4. Honda T, Glass R I, Akhtar Q, Kibriya A K M G: A simple assay to detect *Escherichia coli* producing heat labile enterotoxin: results of a field study of the Biken test in Bangladesh, *Lancet*, 1981; ii: 609–610.
5. March S B, Ratnum S: Sorbitol-MacConkey medium for detection of *Escherichia coli* 0157:H7 associated with haemorrhagic colitis, *Journal of Clinical Microbiology*, 1986; 23: 869–872.
6. WHO: *Guidelines for Drinking Water Quality*, Drinking water quality control in small-community supplies, World Health Organisation, Geneva, 1985; III.

7. Wright R C: A medium for the rapid enumeration of *Escherichia coli* in the presence of other faecal coliforms in tropical waters, *Cambridge Journal of Hygiene*, 1982; 88: 265–273.
8. Cheessbrough M: *Medical Laboratory Manual for Tropical Countries*, Volume III: Microbiology, Butterworh and Company Ltd (publishers,) 1984.
9. Lindskog R U M, Lindskog P A: Bacteriological contamination of water in rural areas: an intervention study from Malawi, *Journal of Tropical Medicine and Hygiene*, 1988; 91: 1–7.
10. El Attar L, Gawad A A, Khairy A E M, El Sabaie O: The sanitary condition of rural drinking water in a Nile Delta village: II, Bacterial contamination of drinking water in a Nile Delta village, *Cambridge Journal of Hygiene*, 1982; 88: 63–67.
11. Echeverria P, Seriwatana J, Taylor D N, Yanggratoke S, Tirapat C: A comparative study of enterotoxigenic *Escherichia coli*, *Shigella*, *Aeromonas* and *Vibrio* as aetiologies of diarrhoea in northeastern Thailand, *American Journal of Tropical Medicine and Hygiene*, 1985; 34: 547–554.
12. Echeverria, P, Taylor D N, Seriwatana J, Leksomboon U, Chaicumpa W, Tirapat C, Rowe B: Potential sources of enterotoxigenic *Escherichia coli* in homes of children with diarrhoea in Thailand, *Bulletin of the World Health Organisation*, 1987; 65: 207—215.