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Commercial milk products and indigenous weaning foods in a rural West African environment: a bacteriological perspective

By R. A. E. BARRELL* and M. G. M. ROWLAND†
Medical Research Council Dunn Nutrition Unit, Keneba Laboratories, The Gambia

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SUMMARY

Two commercially available baby milks, one 'biologically acidified', the other 'non-acidified', and a traditional weaning food, millet gruel, were prepared and stored under village conditions in West Africa. Increases in total colony count and in number of *Bacillus cereus*, *Clostridium welchii*, *Staphylococcus aureus* and *Escherichia coli* were determined in these products when stored as commonly practised at ambient temperatures over a period of 8 h. Poor hygiene during preparation was indicated by readily detectable numbers of coliforms and *E. coli* in freshly prepared samples of each of the milks, though the cooked local gruel seemed less vulnerable in this respect. The rate of increase in the numbers of these organisms was lower in the acidified milk when prepared with unboiled water containing high numbers of coliforms and *E. coli*. Increases in total colony count and in numbers of *Staph. aureus* were also less marked in the acidified milk. When food was not eaten soon after preparation the problem of bacterial overgrowth was as great with the local gruel as with the considerably more nutritious reconstituted milks.

INTRODUCTION

Gastroenteritis is the most important cause of morbidity and mortality in infants and young children in many areas of the developing world (Elliott, 1976). The importance of weanling diarrhoea in infants in these areas is well recognized (Scrimshaw, Taylor & Gordon, 1968) and it is not surprising that dangers inherent in the use of infant formulas in this context have been much publicized (Jelliffe & Jelliffe, 1978). Unfortunately relatively little attention has been directed to the problems of hygiene associated with local weaning foods.

Keneba is a traditional West African village where it is universal practice to breast feed infants well into the second year of life and the first weaning food, usually introduced between 3 and 6 months of age, is a cooked cereal gruel.

Because of poor environmental hygiene local weaning foods are commonly

* Present address: Public Health Laboratory, Withington Hospital, Manchester M20 8LR.
† Present address: Medical Research Council Dunn Nutrition Unit, Dunn Nutritional Laboratory, Milton Road, Cambridge CB4 1XJ.

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overgrown with bacteria to an extent which is almost certainly hazardous to health (Barrell & Rowland, 1979*a*). Thus, despite the absence of commercial infant food products, there is a high prevalence of diarrhoeal disease in infancy with a disastrous effect on growth (Rowland, Cole & Whitehead, 1977; Cole & Parkin, 1977). Moreover the traditional infant foods have a very poor nutritive value.

This study was carried out to determine the bacteriological fate of two commercial milk products compared with that of the traditional weaning food when prepared in the same village environment at two different levels of hygiene.

MATERIALS AND METHODS

Environment

Keneba is an isolated rural subsistence farming community of about 900 persons well described by Thompson (1965) and Tully (1978). Water is obtained from six village wells which are contaminated (Barrell & Rowland, 1979*b*) and some degree of water shortage is always experienced during the annual dry season. Sanitation is virtually non-existent. Food is prepared up to 3 times per day, but particularly during the rainy season when mothers have a heavy farming commitment, food may be prepared in quantities to meet the day's requirements and may thus be stored after cooking as long as 12 h before consumption.

Foods

Pelargon was chosen as a 'biologically acidified' cow's milk preparation, containing lactic acid and viable cells of *Streptococcus lactis*. This was claimed to confer better keeping qualities and even an initial bactericidal effect (Rosachino, 1975; Midulla, Lo Russo & Sabatino, 1976).

Beba was chosen as a non-acidified milk (i.e. not containing lactic acid or *Strep. lactis*) of otherwise similar constitution to *Pelargon*.

A local millet gruel (*Sanyo mono*), one of the commonest early weaning foods usually introduced between 3 and 6 months of age, was also studied to help put into perspective the findings related to the above milk products.

Preparation

Each kind of food was prepared and stored on three occasions by two village housewives. Approximately 400 ml portions were constituted in keeping with usual village practice. The housewives handled the food employing two different standards of hygiene, though in both cases the technique used was representative of normal village practice.

Milks were prepared with 400 ml of water, the quantities of powder used being those recommended by the manufacturer. Housewife A reconstituted the milks using unboiled water and this was stored in an open calabash (gourd bowl) kept off the ground to avoid spillage and gross contamination from passing animals.

Housewife B used boiled water which had been allowed to cool in a clean metal bowl which was then covered and stored off the ground.

Table 1. *The bacteriology of one sample of millet gruel during preparation, cooking and subsequent storage at ambient temperature* 28–37 °C

	Total aerobic colony count/g at 37 °C	Coliforms: presence/absence in 0·1–0·00001 g (g)	*E. coli*: presence/absence in 0·1–0·00001 g (g)
Unpounded grain	$6·6 \times 10^3$	NI	NI
Grain after first pound	$1·3 \times 10^5$	0·001	0·1
After wash	$5·3 \times 10^4$	0·001	0·001
After second pound and sieve	$3·6 \times 10^4$	0·1	0·1
Paste before cooking	$5·0 \times 10^4$	0·0001	0·0001
Cooked millet gruel			
0 h	$1·7 \times 10^3$	0·001	0·001
1 h	$4·0 \times 10^3$	0·01	0·01
2 h	$6·9 \times 10^4$	0·001	0·001
4 h	$3·6 \times 10^6$	0·0001	0·0001
8 h	$7·3 \times 10^8$	0·00001	0·00001
Water used for preparation	$3·3 \times 10^4$/ml	$3·5 \times 10^3$/100 ml*	$1·7 \times 10^3$/100 ml*

* Counts expressed as most probable numbers (MPN)/100 ml. NI, not isolated.

The millet gruel was prepared in the traditional fashion by first winnowing the grain which was then damped with a little unboiled water and pounded using a local wooden pestle and mortar. It was then washed to remove the chaff, repounded and sieved. The resultant moist flour was then mixed with a little cold water to form a thin paste.

Housewife A added this mixture to warm water and continued heating until boiling apparently occurred, followed by simmering for 5 min.

Housewife B mixed the paste with boiling water and simmered for 10 min.

In both cases temperatures were unevenly distributed in the bulk of the gruel, falling in the range of 80–95 °C.

Each housewife then stored the gruel using the same method as for their respective milk preparations.

Sampling

Bacteriological examination was carried out on the samples of boiled and unboiled water used for reconstituting the milk products.

In addition, for one of the *Sanyo mono* samples prepared by housewife B specimens were taken before and after cooking, as shown in Table 1. A sample of water used in the pre-cooking preparation was also examined.

The three kinds of foods were then examined at 0, 1, 2, 4 and 8 h after preparation, time 0 for the gruel being taken as the time at which the food had cooled sufficiently for consumption (40–50 °C).

Maximum and minimum ambient temperatures were measured throughout the 8 h storage periods.

Bacteriological methods

Reconstituted milk products and millet gruel

The total aerobic colony count at 37 °C and counts of *Staph. aureus*, *B. cereus* and *Cl. welchii* were made. Ten g of sample was suspended in 90 ml of quarter-strength Ringer's solution and blended in an Atomix blender (M.S.E.). Tenfold dilutions were prepared and colony counts made by a method modified from that described by Miles & Misra (1938). The presence of *E. coli* in at least 0·1 g was determined in all samples and of *Salmonella* in 25 g samples at 8 h only. Methods were based on those described by Thatcher & Clark (1968) for the enumeration and confirmation of *E. coli*, *B. cereus* and *Cl. welchii*. An organism was designated *E. coli* if it produced gas in brilliant green bile broth (2% Oxoid) and indole in tryptone water (Oxoid) both incubated at 44 °C. Similarly standard methods were also used for the confirmation of *Staph. aureus* and *Salmonella*. Colony counts of *Staph. aureus* were made on Kranep agar (Oxoid) and the total aerobic colony count on plate count agar (PCA-Oxoid) incubated at 37 °C for 48 h.

The presence of *Salmonella* was determined by the enrichment of 25 g samples in 100 ml Müeller–Kaufmann tetrathionate broth (Oxoid) incubated at 41 °C for 24 h, followed by subculture on to deoxycholate citrate agar (Hynes modification) (Oxoid). Suspect colonies were subcultured into Kohn's media I and II (Oxoid). Cultures giving the characteristic biochemical reactions for *Salmonella* were tested for agglutination with *Salmonella* polyvalent H-specific and non-specific antiserum (Burroughs Wellcome).

Foods and milks before reconstitution

The total colony count at 37 °C and counts for *Staph. aureus*, *B. cereus* and *C. welchii* were made as already described. The presence of coliform organisms was determined in five 1 g samples each inoculated into 10 ml of McConkey broth (Oxoid). The cultures were examined for acid and gas production after incubation at 37 °C for 24 and 8 h. The presence of *Salmonella* was determined in 25 g samples by suspension in 225 ml buffered peptone water (Edel & Kampelmacher, 1973) followed by pre-enrichment at 37 °C for 24 h. A 1 ml subsample of this culture was transferred to 10 ml single strength Müller–Kauffmann tetrathionate broth followed by incubation and plating as described for foods. The two milk powders and a pounded sample of millet prepared by each housewife were examined.

Water samples

The water used for reconstitution of the baby milks was examined for the presence of coliforms, *E. coli* and *Salmonella*. The water used by housewife B in the preparation of the millet gruel was also examined for total colony count at 37 °C. The most probable number (MPN) of coliforms and *E. coli* was determined in 100 ml water using minerals modified glutamate medium (Oxoid) containing Durham tubes. The samples were incubated at 37 °C and examined for

acid and gas production indicative of coliforms at 24 and 48 h. The presence of *E. coli* in these cultures was determined in the manner described above for foods. The total aerobic colony count was made on PCA as described for foods.

The presence of *Salmonella* was determined in 100 ml water by adding an equal volume of double strength Müller–Kauffmann tetrathionate broth followed by incubating at 41 °C for 24 h. Plating and confirmation were as described above.

RESULTS

The bacteriology of milk powder and cereal flour before reconstitution or cooking

In both milk powders, the colony count of all organisms sought was less than 3×10^2/g. Coliforms were not detected in a 5 g sample of powder and *Salmonella* was not detected in 25 g. A sample of millet flour prepared by housewife A immediately before water was added to make a paste had a total aerobic colony count of 9×10^8/g, and *E. coli* was detected in 0·01 g. Table 1 shows the numbers of bacteria detected at each stage in preparation of a sample examined from housewife B. The flour before cooking had a total aerobic viable count of $3{\cdot}6 \times 10^4$/g and *E. coli* was detected in 0·1 g. The water used for the preparation of this sample had an MPN of $1{\cdot}7 \times 10^3$/100 ml *E. coli*. *Salmonella* was not detected in 100 ml water. The addition of water to the flour was associated with a considerable increase in the total aerobic colony count and in the numbers of coliforms, and *E. coli*., *Staph. aureus* and *Cl. welchii* were not detected in this sample.

The bacteriology of water used for reconstitution of milk

As shown in Table 2, for unboiled water MPN values of *E. coli* obtained at 8 h varied from $1{\cdot}7 \times 10^3$ to $1{\cdot}1 \times 10^4$/100 ml.

Cooked millet gruel and reconstituted milks

The minimum and maximum counts of all organisms sought after storage for 8 h are also shown in Table 2.

B. cereus was not isolated from any sample of acidified milk but was found in one sample of non-acidified milk prepared with unboiled water, and increased from 3×10^2/g at 2 h to 6×10^2/g at 4 h and 9×10^4/g at 8 h. In the non-acidified milk prepared with boiled water it was not detected in any sample in the 0–4 h period but was isolated from each of these samples prepared with boiled water after storage for 8 h. Counts were in the range 1·6 to $5{\cdot}6 \times 10^5$/g. The counts of *B. cereus* in the millet gruel at 8 h are shown in Table 2. Counts in excess of 3×10^2/g did not occur before 8 h.

Staph. aureus was not isolated from any sample of food prepared by housewife A. It was detected in one sample of acidified milk prepared with boiled water at 8 h and one sample of millet gruel prepared by housewife B at 8 h (Table 2). It was also detected in 2 samples of non-acidified milk prepared with boiled water after storage for 8 h with counts of $9{\cdot}0 \times 10^3$/g and $1{\cdot}6 \times 10^4$/g.

Cl. welchii was detected in 2 samples of millet gruel prepared by housewife B after storage for 8 h. The counts were $3{\cdot}3 \times 10^2$/g and $3{\cdot}4 \times 10^4$/g.

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Table 2. *Maximum and minimum counts of bacteria in millet gruel and reconstituted milks after 8 h storage, and in the water used in the reconstitution of the milks*

		Millet gruel*		Acidified milk*		Non-acidified milk*		Water for milks†	
		Housewife A	Housewife B	Unboiled water	Boiled water	Unboiled water	Boiled water	Unboiled	Boiled
B. cereus	Min	NI	NI	NI	NI	NI	$2{\cdot}0 \times 10^5$	—	—
	Max	$7{\cdot}0 \times 10^3$	$1{\cdot}2 \times 10^5$	NI	NI	$9{\cdot}0 \times 10^4$	$5{\cdot}6 \times 10^5$	—	—
Staph. aureus	Min	NI	NI	NI	NI	NI	NI	—	—
	Max	NI	$6{\cdot}0 \times 10^2$	NI	$6{\cdot}0 \times 10^2$	NI	$1{\cdot}6 \times 10^4$	—	—
Cl. welchii	Min	NI	NI	NI	NI	NI	NI	—	—
	Max	NI	$3{\cdot}4 \times 10^4$	NI	NI	NI	NI	—	—
Coliforms	Min	0·1 g	0·00001 g	0·0001 g	0·00001 g	0·00001 g	0·00001 g	$5{\cdot}5 \times 10^4$/100 ml	< 1/100 ml
	Max	0·00001 g	0·00001 g	0·00001 g	0·00001 g	0·00001 g	0·00001 g	$9{\cdot}0 \times 10^4$/100 ml	< 1/100 ml
E. coli	Min	NI	0·1 g	0·001 g	0·00001 g	0·00001 g	0·0001 g	$1{\cdot}7 \times 10^3$/100 ml	< 1/100 ml
	Max	0·00001 g	0·00001 g	0·00001 g	0·00001 g	0·00001 g	0·00001 g	$1{\cdot}1 \times 10^4$/100 ml	< 1/100 ml
Salmonella in 25 g		NI	NI	Present in 1 out of 3 samples	NI	NI	NI	NI	NI
Total viable count at 37 °C	Min	$2{\cdot}3 \times 10^6$	$5{\cdot}0 \times 10^7$	$1{\cdot}1 \times 10^6$	$3{\cdot}0 \times 10^5$	$9{\cdot}0 \times 10^7$	$1{\cdot}7 \times 10^8$	—	—
	Max	$1{\cdot}2 \times 10^8$	$7{\cdot}3 \times 10^8$	$9{\cdot}1 \times 10^7$	$2{\cdot}3 \times 10^7$	$4{\cdot}0 \times 10^8$	$7{\cdot}6 \times 10^8$	—	—

* In millet gruels and milks counts of coliforms and *E. coli* are expressed as presence in 0·1–0·00001 g, *Salmonella* as presence in 25 g and other organisms as counts per g.

† In water, counts of coliforms and *E. coli* are expressed as MPN/100 ml and *Salmonella* as presence in 100 ml.

NI Not isolated.

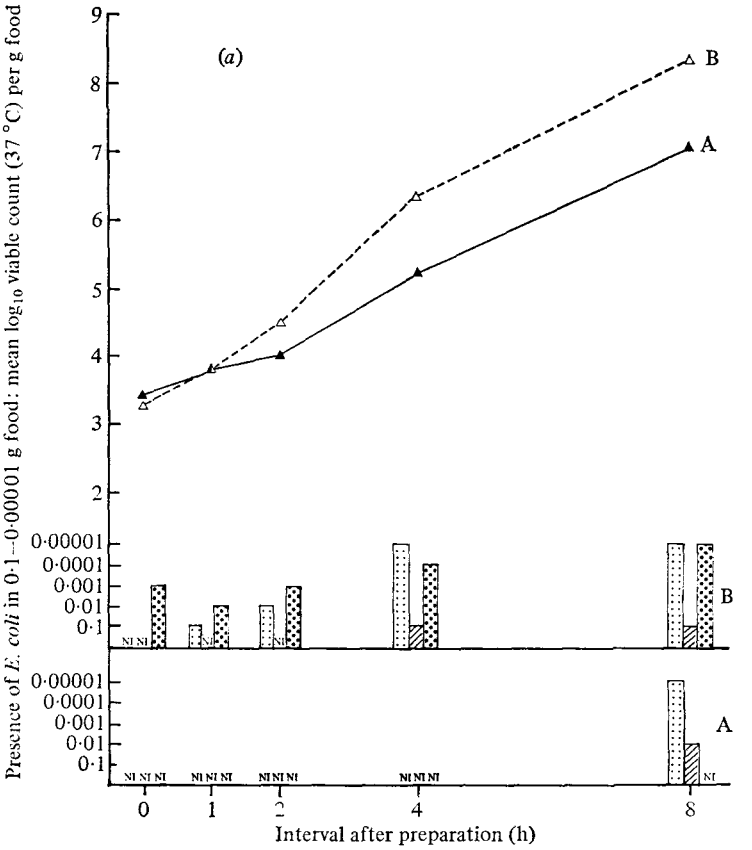

Fig. 1. Log_{10} total aerobic viable counts at 37 °C and presence of *E. coli* in 0·1–0·00001 g food at 0, 1, 2, 4 and 8 h after preparation. ⊡, ▨, ⊡ indicate detection of *E. coli* in three separate experiments for each food examined. Values for total viable count are the geometric mean for the 3 experiments.

Fig. 1 (*a*). Millet gruel. Mean total viable count in 3 samples prepared by housewife A, —▲—, and housewife B, —△—.

The counts of coliforms and *E. coli* obtained after 8 h storage in the different samples are shown in Table 2. Total colony counts and *E. coli* counts throughout the 8 h storage periods for all foods are shown in Fig. 1. Higher counts were generally observed in the millet gruel prepared by housewife B than that prepared by housewife A (Fig. 1*a*). For milks prepared with boiled water there was little difference in the numbers of *E. coli*. When unboiled water was used, however, and initial numbers of *E. coli* were high, particularly in the acidified milk, there was noticeably less multiplication, and by 8 h counts were higher in the non-acidified milk (Fig. 1*b*, *c*).

Total aerobic colony counts are also shown in Fig. 1. The rate of multiplication of bacteria isolated aerobically on PCA at 37 °C was lower in the acidified milk than in the non-acidified milk, irrespective of whether boiled or unboiled water was used for preparation. Similarly, the rate of multiplication was lower in acidified milk than in millet gruel.

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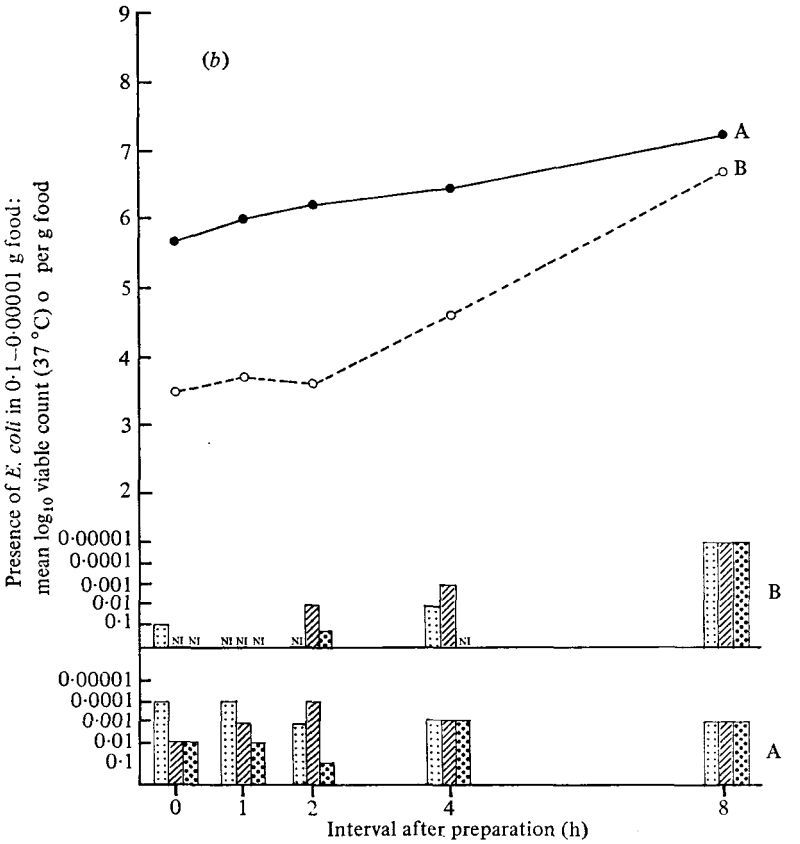

Fig. 1 (*b*). Acidified milk. Mean total viable count in three samples prepared with unboiled water (A) —●—, and boiled water (B) – – – ○ – – –.

Salmonella was detected in one sample only of acidified milk prepared with unboiled water after storage for 8 h.

Ambient temperatures during storage of samples from housewife A were in the range 28–31 °C (millet gruel and milks prepared with unboiled water) and 28–37 °C during storage of those prepared by housewife B.

DISCUSSION

The factors affecting the bacteriological quality of the freshly prepared milk and gruel were the degree of contamination in the water and the domestic utensils, the personal hygiene of the food handler and, in the case of the local foods, the contamination in the cereal flour itself.

The village well water in Keneba was heavily contaminated, counts of faecal coliforms often exceeding 10^4/100 ml. This was an important source of bacteria present in milk prepared with unboiled water, in the pounded millet which had been dampened during pounding and in the washed pots and spoons used during preparation. Metal bowls scrubbed with soap have been found to contain up to 10^5–10^6 viable bacteria per bowl (Barrell, unpublished data, see Appendix) and

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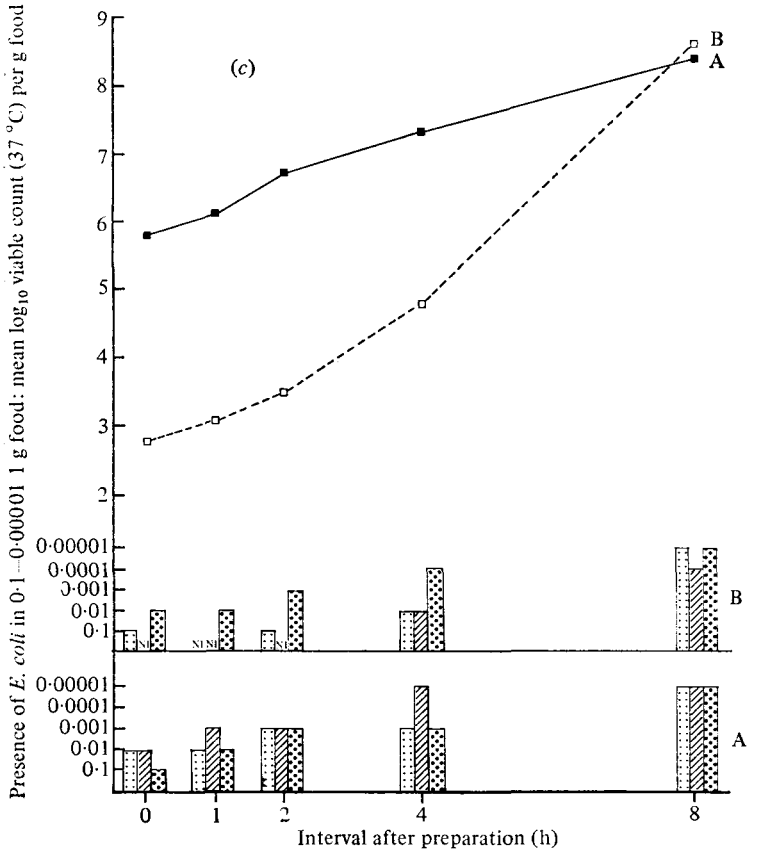

Fig. 1(*c*). Non-acidified milk. Mean total viable count in three samples prepared with unboiled water (A) —■—, and boiled water (B) ---□---.

one bowl contained at least 10^2 coliforms and *E. coli*. Bacteria present on the utensils were distributed throughout milks during stirring and preparation and *E. coli* were initially detectable in less than 0·1 g in some of the milks prepared, even when they were reconstituted with boiled water.

The use of unboiled water in the preparation of the millet gruel (*Sanyo mono*) was associated with an increase in total aerobic viable count and an input of *E. coli* (Table 1). Similar observations were made on the preparation of tortillas by Capparelli & Mata (1975), who concluded that most of the initial contamination was introduced by water and hands. The cooking of the millet gruel resulted in a decrease in numbers of *E. coli*, although these were still detected at up to 10^3/g in the freshly cooked product. The gruel prepared by housewife A generally had a higher temperature after cooking and was of better bacteriological quality than that prepared by housewife B (Fig. 1*a*). Poor heat penetration was evident in the gruels prepared by both housewives, as indicated by uneven temperature distribution. In this instance the cooking method seemed more important than any other aspect of domestic hygiene. However, prolonged cooking results in a product that is gelatinous and difficult for the young infant to ingest. After

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preparation of milks and gruels was complete two factors appeared to determine the degree of bacterial overgrowth. Clearly the greater the interval between food preparation and its consumption, the greater the numbers of bacteria that were present. The nature of the food also seemed important in that the biologically acidified milk did appear to exert a mild bactericidal effect when the initial contamination was heavy. Apart from this all the preparations seemed equally susceptible to bacterial overgrowth.

In general, the counts of *Staph. aureus*, *Cl. welchii* and *B. cereus* at 8 h were usually lower than those commonly associated with food poisoning (Thatcher & Clark, 1968). However, the age and the nutritional status of the children who would consume these foods almost certainly put them in an 'at risk' group, and the stricter recommendations of the International Commission on Microbiological Specifications for Foods (ICMSF 1974) for special dietary food appear more appropriate. The period when supplementary weaning foods are introduced in Keneba coincides with an increase in the incidence of diarrhoea and impaired growth performance in these children (Rowland, Barrell & Whitehead, 1978). The use of commercial baby milks in developing countries has been a subject of much controversy in recent years and it has been suggested that the risk of diarrhoeal disease is greater with the consumption of these milks than with locally produced foods. Our results suggest that both types of foods can act as a vehicle for the transmission of diarrhoeal disease. The unhygienic preparation of milks and the inadequate cooking of gruels can both result in a product which is unsafe even at the time of preparation. The hazard is enhanced by prolonged storage of the preparation at ambient temperatures before consumption, a practice common in developing countries (Barrell & Rowland, 1979*a*). As long as it is necessary to supplement the diet of the young infant it is desirable on nutritional grounds that at least part of that food supplement should be milk (Whitehead, 1976), either from local or outside sources. 'Biological acidification' of milk may confer some protection to the hygienic qualities of the commercial product but there is a real need to seek further improvement in this field. The provision of safe water supplies would also be expected to be beneficial.

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APPENDIX

Unpublished data quoted in attached paper submitted to the Journal of Hygiene.

Bacteriological contamination of food containers

The bowls were swabbed with absorbent cotton wool swabs (*ca.* 5 cm in diameter). Two swabs were used for each bowl. The first was soaked in $\frac{1}{4}$-strength Ringer's solution ($\frac{1}{4}$R) taken from a container holding 2 dl of this solution. The swab was rubbed over the interior surface of the bowl and returned to the container plus $\frac{1}{4}$R. The interior surface of the bowl was then rubbed dry with the second swab (which was not moistened) and the swab transferred to the container with the first swab. 0·1 ml of this suspension was plated on PCA (Oxoid) and incubated at 37 °C for 2 d. In addition, for 3 bowls, 1 ml of suspension was incubated in McConkey broth for coliforms and further subcultured for detection of *E. coli*.

Results

1. Total colony counts:

Calabashes
A = $8{\cdot}4 \times 10^5$
B = $8{\cdot}0 \times 10^5$
C = $1{\cdot}0 \times 10^6$
D = $6{\cdot}8 \times 10^5$
E = $4{\cdot}0 \times 10^5$

Metal bowls A = $3{\cdot}0 \times 10^5$
B = $9{\cdot}0 \times 10^5$
C = $1{\cdot}4 \times 10^5$
D = $1{\cdot}4 \times 10^5$
E = $2{\cdot}0 \times 10^5$

2. Coliforms, *E. coli* and total colony counts:

Metal bowls F Coliforms – NI
E. coli – NI
Total colony count = $3{\cdot}6 \times 10^5$
G Coliforms and *E. coli* – NI
Total colony count = $3{\cdot}0 \times 10^5$
H Coliforms and *E. coli* = $2 \times 10^2 - 2 \times 10^3$
(present in 1 ml suspension)
Total colony count = $6{\cdot}6 \times 10^5$

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