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Enteropathogenic bacteria in faecal swabs of young children fed on lactic acid-fermented cereal gruels

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SUMMARY

The influence of consumption of a lactic acid-fermented cereal gruel *togwa* with pH $\leqslant 4$ on the presence of faecal enteric bacteria such as campylobacter, enterohaemorrhagic *Escherichia coli* (EHEC:O157), enterotoxigenic *Escherichia coli* (ETEC), salmonella and shigella was evaluated. Under 5 years old healthy children listed in an ascending order of age were alternatively assigned and given either a lactic-acid fermented cereal gruel *togwa* (test diet) or an unfermented cereal gruel *uji* (control diet) once a day for 13 consecutive days. The presence of the enteropathogens was examined in rectal swabs collected from the children at baseline (before feeding session started), on days 7 and 13, and additionally 14 days (follow-up day) after the feeding session had stopped. The swabs were cultured on to different optimal media for respective enteropathogen and confirmed by standard microbiological and serological methods. *Campylobacter* spp. dominated among the enteropathogens (62% out of total) followed by *Salmonella* spp., ETEC and *Shigella* spp. Children with isolated enteropathogens in the *togwa* group was significantly reduced ($P < 0{\cdot}001$) from 27·6% at baseline to 7·8, 8·2 and 12·7% on days 7, 13 and follow-up day, respectively. The effect was more pronounced in those children taking *togwa* > 6 times during the study period. In the control group, there was a slight decrease from 16·7% at baseline to 11·4% on day 7 and 8·1% on day 13. On the follow-up day, enteropathogens were found in 22·6% of the children, which was significantly higher than in those children taking *togwa* > 6 times. We conclude, that regular consumption of *togwa* with pH $\leqslant 4$, once a day, three times a week may help to control intestinal colonization with potential diarrhoea-causing pathogens in young children.

INTRODUCTION

Campylobacter jejuni/coli, enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *Escherichia coli* (ETEC), *Salmonella* spp., *Shigella* spp. and rotavirus are consistently implicated with diarrhoea in under 5 years old children in developing countries [1–7]. Other agents causing diarrhoea in children but to a lesser extent are *Vibrio cholera 01* [1] and enterohaemorrhagic *Escherichia coli* (EHEC:O157) [7]. In a former aetiology study in the Southern part of Tanzania (unpublished observations), we isolated *Campylobacter* sp., ETEC, *Salmonella* sp. or *Shigella* sp. in stools from 43% of children with diarrhoea symptoms and 32% from the asymptomatic children. Our findings were in accord with those by Lindblom and colleagues [8] who reported campylobacter and

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ETEC to be present in 18 and 20%, respectively, of young children with diarrhoea in North West of Tanzania. The corresponding proportions among children without diarrhoea were 15 and 5%, respectively. These results indicate that a high proportion of diarrhoea among children in the studied areas is of bacterial origin and that many asymptomatic children are carriers of enteropathogens. The colonization with these potential diarrhoea-causing bacteria may predispose asymptomatic young children to develop diarrhoea disorders [7]. It is therefore imperative to protect young children from being colonized with these diarrhoea-causing bacteria.

Cereal gruels that are the main complementary foods in Tanzania are sometimes prepared to last for several meals over the day. Due to poor hygienic conditions prevailing in most households of Tanzania, these complementary foods may frequently be contaminated with enteropathogens, especially when stored at high ambient temperatures. Such findings have been reported from Peru [2], Bangladesh [9] and Nigeria [10].

Consumption of lactic acid bacteria (LAB)-fermented foods is believed to be beneficial in a number of ways including a therapeutic and prophylactic effect against diarrhoea disorders. Numerous studies have shown that, lactic acid-fermented foods suppress the growth of enteropathogenic bacteria [11–14] and control intestinal colonization with enteropathogenic bacteria or diarrhoeal infections [15–20].

Togwa is a cereal based lactic acid-fermented beverage widely used as a soft drink for all age-groups in Tanzania as well as a complementary food in some parts of the country [21]. *Togwa* with pH $\leqslant 4$ is able to suppress the growth of enteropathogenic bacteria and production of enterotoxins [22–24], lower the number of diarrhoea episodes among regular consumers [25] and improve the intestinal permeability during acute diarrhoea [26].

This study assessed the presence of enteropathogenic bacteria such as campylobacter, EHEC:O157, ETEC, salmonella and shigella in rectal swabs of children regularly feeding on *togwa*.

MATERIALS AND METHODS

Study area

The study was conducted in Majohe village situated approx. 30 km southwest of Dar-es-Salaam in Tanzania. The climate is hot (28–33 °C) and humid throughout the year. Long rains come between March and June and short rains during September and October. Clinical records obtained from the village health centre showed diarrhoea to be one of the major causes of morbidity in the village. *Togwa* is well known and is extensively consumed during the dry hot season as a refreshment. *Togwa* is also given to young children, and is normally available throughout the year.

Study design and objectives

The study was an experimental controlled feeding study including children aged 6–60 months. It was conducted from November to December 1995 (dry, hot season). The objective was to investigate the presence of enteropathogenic bacteria in rectal swabs of children fed once per day either a lactic acid-fermented cereal gruel, *togwa*, (test diet) or a non-fermented cereal gruel, *uji* (control diet). Four nutritionists supervised the feeding session in a day-care centre during 13 consecutive days. They also administered a 24-h questionnaire on the health and home feeding patterns of the children on each feeding day. Rectal swabs, for the identification of enteropathogens, were collected from the children by a team of three clinical microbiologists. Swabs were collected at baseline (before feeding session had started), during the feeding period (day 7 and 13 after the feeding session had started) and after intervention (14 days after the feeding session had stopped). The pH of *togwa* and *uji* was assessed daily using a portable pH-meter. The study was approved by the Tanzania Food and Nutrition Center's Research and Ethics Committee, and an oral informed consent was obtained from mothers or caretakers before the study begun.

The study was preceded by a baseline survey during which potential confounding factors such as age, sex and anthropometric measurements of the children were recorded. Age of children was obtained from the Maternal and Child Health (MCH) card. The health of the children and whether they had received any treatment for any illness within 2-weeks prior to the baseline survey was recorded. Information about child feeding practices (24 h recall) including breast-feeding, pre-treatment of the food and water to drink given to children as well as hygienic practices on child food preparation and handling by the mother was also collected.

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Table 1. *Group assignment to diets and children attendance during the entire study period*

	Togwa 1	*Togwa* 2	Control	Total
Number of children assigned at baseline	50	50	51	151
Excluded from data analysis because they				
Dropped out after baseline faecal swabs	6	7	None	13
Mixed diets during feeding session	n.a.*	n.a.	9	9
Number of children available for analysis	44	43	42	129
Number of children with isolated enteropathogens at baseline†	10 (23)	13 (30)	7 (17)	30 (23)
Number of children whose faecal swabs were collected during†				
Four sampling occasions	19 (43)	27 (63)	22 (52)	68 (53)
Three sampling occasions	21 (48)	12 (28)	17 (41)	50 (39)
Two sampling occasions	4 (9)	4 (9)	3 (7)	11 (9)
Intake frequency of *togwa* as of day 13 (mean ± S.D.)	5·4 ± 3·0	6·2 ± 3·3	n.a.	—

* Not applicable.
† Figures within parentheses are percentage out of total number of children included in the data analysis.

Assignment of the children to diet groups

One list of a total of 151 children, who were eligible for the study (6–60 months old) and registered during the baseline survey, was prepared in an ascending order of age. From the list, children were alternatively divided into three groups. The first was assigned to a lactic acid-fermented maize gruel, *togwa*, prepared using a starter culture (*sc* 1) developed by daily recycling *togwa* collected from the study village, hereafter referred to as test diet 1 (*togwa* 1). The second group was assigned to another lactic acid-fermented maize gruel, test diet 2 (*togwa* 2), prepared using a starter culture (*sc* 2) of a *Lactobacillus plantarum* (299v) kindly provided by G. Molin from the University of Lund, Sweden. This lactobacillus strain is known to colonize the human gut [27]. The third group was assigned to an unfermented maize gruel *uji* (control diet, Table 1).

Sample size

The sample size was calculated based on findings from a previous aetiology study (unpublished observation) that 32% of children without diarrhoea symptoms were colonized with enteropathogens. Assuming that the rate of response in the control group was 5% and 75% in the study group, the sample size at baseline should be at least 50 children for each group. The confidence limit was 95% with 80% power and the ratio of exposed to non-exposed children was estimated to be 1:1 [28].

Since the study was conducted during the dry season, fewer children than expected were detected with enteropathogens at baseline (Table 1). To increase the number of children colonized with enteropathogens at baseline, the two *togwa* groups were eventually combined during data analysis. Moreover, the two starter cultures (*sc* 1 and *sc* 2) produced *togwa* with similar pH and the mean intake frequency of *togwa* as of day 13 for children assigned to *togwa* 1 and *togwa* 2 was also similar (Table 1).

Socio-economic characteristics of the households and participating children at baseline

Table 2 shows the characteristics of households and participating children at baseline in the *togwa*- and control group, respectively. Apart from the significantly larger proportion of boys (54%) in the *togwa* group compared with the proportion (31%) in the control group ($P < 0{\cdot}01$) and that the median age for stopping breast-feeding (24·3 months) in the *togwa* group was relatively lower ($P < 0{\cdot}02$) compared with control group (26·5 months), the rest of the possible confounders were equally distributed between the two groups. Of the 50 children aged 6–24 months, 47 were still breast-feeding on demand and bottle feeding was non-existent.

Gruel preparations

Togwa was prepared by mixing ungerminated maize flour with boiling water (10% w/v). The mixture was further cooked for 20 min to a gruel and then cooled to approx. 35 °C. Power flour (*pf*) prepared from germinated millet *Eleusine coracana* (*L*) [22] was then added at a rate of 5% (w/w of flour) in combination

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Table 2. *Characteristics of households and participating children at baseline**

	Togwa ($n = 87$)	Control ($n = 42$)	*P*-value
Child characteristics			
Percentage of males	54	31	0·01
Mean age of children (months ± S.D.)	29·0 ± 14·5 (61)	25·4 ± 12·9 (37)	n.s.
Weight for age, *z* score (mean ± S.D.)	−1·1 ± 1·0 (56)	−1·2 ± 1·1 (36)	n.s.
Age of children (months ± S.D.) with isolated enteropathogens	22·8 ± 11·9 (17)	15·1 ± 9·4 (7)	n.s.
Children (%) with			
fever[a]	9·0	9·5	n.s.
diarrhoea[a]	2·2	0·0	n.s.
Children's feeding pattern (%)			
Still breast-feeding	33·3	50·0	n.s.
Given rice/thick porridge or plain thin gruel[b]	93·2	88·0	n.s.
Given fruits/fruit juices[b]	71·9	59·0	n.s.
Given home-made *togwa*[c]	23·9	14·3	n.s.
Median age for stopping breast-feeding (months)	24·3 (60)	26·5 (41)	0·02
Characteristics of households (%)			
Parents/care takers who are farmers	95·2	89·7	n.s.
Mothers who can read and write	64·3	64·1	n.s.
Father who can read and write	86·1	94·7	n.s.
Households with relatively good/strong houses†	40·0	55·3	n.s.
Households with nonventilated pit latrine	97·5	97·4	n.s.
Households getting water from open wells	96·4	92·5	n.s.
Households with relatively good hygienic practices‡	71·4	60·0	n.s.
Mean ± S.D.			
Age of mother of child (years)	26·8 ± 7·8 (58)	28·1 ± 7·8 (29)	n.s.
Household size	6·5 ± 2·7 (84)	5·9 ± 2·4 (40)	n.s.
Number of < 5 years old children	2·0 ± 1·0 (84)	1·7 ± 0·8 (39)	n.s.
Number of cooking pots	5·9 ± 3·8 (84)	5·6 ± 1·9 (40)	n.s.
Number of water pots	1·0 ± 0·7 (76)	1·1 ± 0·7 (26)	n.s.
Amount of water collected/day (litres)	67·3 ± 38·7 (83)	67·3 ± 28·5 (39)	n.s.
Number of livestocks (chicken)	9·9 ± 13·6 (77)	7·8 ± 8·9 (35)	n.s.
Number of house rooms	3·4 ± 1·3 (84)	3·5 ± 1·8 (39)	n.s.

* Figures within parentheses are number of households/children assessed. [a] Within 2 weeks prior to the study. [b] 24-h recall method. [c] Within 1 week prior to the study.
† House with block/brick walls, concrete floors, iron sheet roofs and/or wooden doors.
‡ Reboil left over food/water to drink; and utensils, mother's and child's hands are washed with soap before feeding the child.

with either of the two starter cultures at 10% (v/v). These were produced by daily inoculum recycling of the previous batch of *togwa* 1 or *togwa* 2, respectively [14]. The gruels were then left to ferment in the village at room temperature, 25 °C for 12 h.

Gruel without starters (control gruel, *uji*) was prepared as control gruels in a similar way to the gruels prepared for *togwa* on each feeding day. In addition 10% (w/w) roasted groundnut paste was added to the maize flour to enrich the nutritional quality of the control gruel. Sugar was added to all gruels just before feeding to make them more palatable. The pH of the two *togwa* during the study period ranged between 3·1 and 4·1 ($\bar{x} = 3·7$) and the mean pH of control gruels was 6·3.

Feeding schedule

Each morning at about 09.00 h, for 13 consecutive study days, mothers with their children enrolled in the study, assembled at the day care centre in the village where gruels were centrally prepared and served. The mothers were interviewed on the health and food the child had eaten over the last 24-h including what the child was given at the study centre.

Enteropathogen identification

The rectal faecal swabs were transported in a Stuart transport media [29] to the laboratory and cultivated within 3–4 h for screening of campylobacter,

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EHEC:O157, ETEC, salmonella and shigella. The clinical microbiologists visited the feeding centre on the sampling day to collect the faecal swabs. Swab collection was done in a separate room and the allocation of the diets to the children were anonymous to the microbiologists. This arrangement allowed for blind collection and cultivation of the faecal swabs. Campylobacter was identified from blood-free selective (BFS) media [30], EHEC onto sorbitol-MacConkey-agar, ETEC from Drigalski agar; salmonella and shigella from desoxycholate-citrate (DC) agar and xylose-lysine-desoxycholate (XLD) agar. The BFS agar was incubated in a micro-aerobic atmosphere using candle jars at 42 °C for 48–72 h, and the remaining media were incubated aerobically at 37 °C for 24–48 h. The faecal samples were also enriched in selenite broth for 24 h before sub-cultivating them onto selective and differential media. The bacteria were further confirmed by microscopical identification of campylobacter and standard microbiological and serological methods for EHEC, ETEC, salmonella and shigella respectively. For the identification of heat labile (LT) enterotoxin produced by *E. coli* the Phadebact® ETEC-LT test were used following the method recommended by the manufacturer (Karo-Bio Diagnostic AB, Huddinge, Sweden). Strains of *E. coli* were collected and transported to Sweden for further identification of toxin production (ETEC-LT) and serotyping (*E. coli*:O157) of EHEC. For identification of EHEC, the diagnostic reagents for *E. coli*:O157 latex test DR 620 M, Oxoid were used (Unipath Limited, Basingstoke, Hants, England).

Data analysis

Table 1 shows that the number of dropout children (13) during the study period was fairly low. The table also shows that over 90% of the children who continued in the study were present for three or four faecal sampling occasions. All children appearing for one or more sampling occasions after baseline are included in the data analysis.

A two-sample paired and unpaired t test were performed to compare prevalence of enteropathogens in children within and between groups, respectively, for each sampling day. The analysis was further controlled for most of the potential confounders including breast-feeding and sex (Table 2).

RESULTS

Table 3 presents the distribution of different enteropathogens isolated during the entire study perid. A total of 445 faecal swabs were collected over the four sampling occasions (baseline to follow-up day). Three hundred swabs were from children assigned to the *togwa* group and 145 from the control group. There were a total of 65 (14·6%) swabs with isolated enteropathogens (one child was colonized at one sampling occasion with two different enteropathogens). Campylobacter was found in 42 (9·4%) of the total faecal swabs collected, salmonella in 11 (2·5%), ETEC in 7 (1·6%), shigella in 5 (1·1%) and no EHEC:O157 was detected.

Effect of *togwa* consumption on faecal enteropathogens

Figure 1 shows the percentage of children with isolated faecal enteropathogens on each sampling day. At baseline, enteropathogenic bacteria were isolated from 27·6% of the children in the *togwa* group and 16·7% in the control group. In the *togwa* group, at day 7, and day 13, enteropathogens were present in 7·8 and 8·2% of the children, respectively, and on the follow-up day in 12·7%. These prevalences were significantly lower than that at baseline, using a paired t test ($P < 0{\cdot}001$). In the control group, there was a slight decrease during the study period, to 11·4% on day 7 and to 8·1% on day 13. On the follow-up day, enteropathogens were found in 22·6% of the children. None of these prevalences was significantly different from that at baseline.

In those children who took *togwa* > 6 times ($n = 36$) during the study period, 38·9% had enteropathogens at baseline. On days 7, 13 and follow-up day significantly ($P < 0{\cdot}001$) fewer children had enteropathogens, 6·1, 6·9 and 6·5%, respectively. The prevalence on the follow-up day (6·5%) was also significantly lower ($P \leqslant 0{\cdot}04$) than that in the control group (22·6%) on the same day, using an unpaired t test. For children who took *togwa* < 7 times ($n = 51$), the percentage with isolated enteropathogens was slightly lower on days 7 and 13 (8 and 11·1%, respectively) compared with 20% at baseline and 21·1% on the follow-up day.

Due to the small number of children with isolated enteropathogens at baseline (23 in the *togwa* group, and 7 in the control group) it was not possible to show any significant therapeutic effect of *togwa* in com-

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Table 3. *Enteropathogenic bacteria isolated during the entire study period**

	Togwa	Control	Total
Total swabs assessed	300	145	445
Type and number of enteropathogenic bacteria isolated			
Campylobacter	29 (9·7)	13 (9·0)	42 (9·4)
Salmonella	8 (2·7)	3 (2·1)	11 (2·5)
ETEC	5 (1·7)	2 (1·4)	7 (1·6)
Shigella	2 (0·7)	3 (2·1)	5 (1·1)
EHEC:O157	None	None	None
Total bacteria isolated	44 (14·7)	21 (14·5)	65 (14·6)

* Figures within parentheses are percentage of total faecal swabs examined.

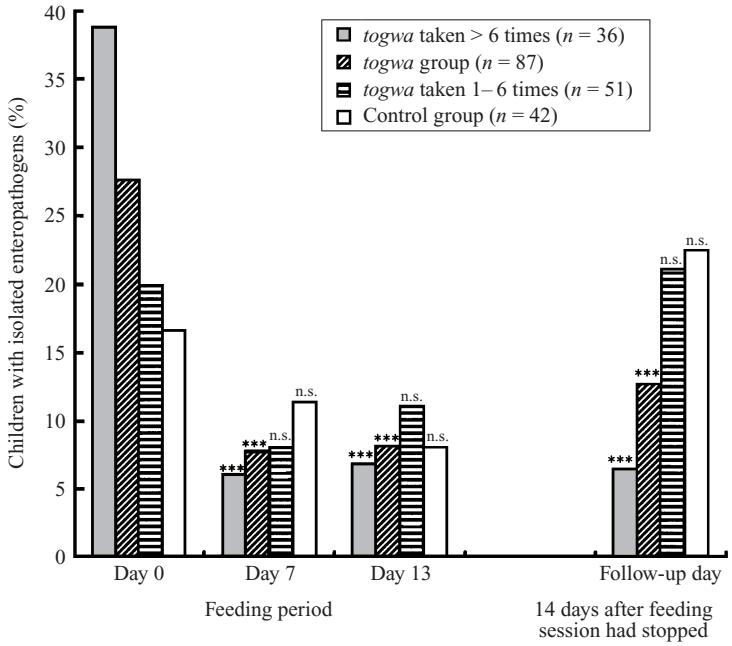

Fig. 1. Percentage of children with isolated faecal enteropathogens in *togwa* and control group, respectively, over the entire study period. ***, significantly different from day 0 ($P < 0{\cdot}001$); n.s., not significant.

Table 4. *Occurrence of enteropathogens in children on days 7, 13 and follow-up day; in those who were free from enteropathogens at baseline**

	Togwa	Control	*P*-value†
Free from enteropathogens at baseline (*n*)	29	19	
Children with isolated enteropathogens (%)			
Day 7	6·9	5·3	
Day 13	3·4	10·5	0·17
Follow-up day	6·9	21·1	0·08

* Children who were examined at all four sampling occasions.
† Unpaired *t* test.

parison with that of the control gruel during the study period and follow-up day. Among the children who were free from enteropathogens at baseline, the percentage of children in the *togwa* group with isolated enteropathogens during the study period and follow-up day was relatively low compared with control children (Table 4, unpaired *t* test). The difference was, however, not significant due to the small number of children in both groups being colonized with enteropathogens.

Effect of confounders and modifiers

Of the possible confounders included in this study, mean age of stopping breast-feeding and sex differed

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Table 5. *Prevalence of enteropathogens in faecal swabs in children from* togwa *and control group* (%). *Stratified by breast-feeding and sex*†

	Baseline	Day 7	Day 13	Follow-up day‡
Breast-feeding				
Togwa	37·9 (29)	7·4 (27)***	12·5 (24)**	21·1 (19)
Control	28·6 (21)	5·3 (19)*	11·8 (17)	31·3 (16)
Non-breast-feeding				
Togwa	22·4 (58)§	8·0 (50)**	6·1 (49)**	9·1 (44)**
Control	4·8 (21)	18·8 (16)	5·0 (20)	13·3 (15)
Males				
Togwa	34·0 (47)§	9·1 (44)**	9·3 (43)**	13·9 (36)**
Control	7·7 (13)	0·0 (12)	9·1 (11)	25·0 (12)
Females				
Togwa	20·0 (40)	6·1 (33)*	6·7 (30)*	11·1 (27)
Control	20·7 (29)	17·4 (23)	7·7 (26)	21·1 (19)

Figures in parentheses are total number of children assessed.
† Values in the same row followed by star superscripts are significantly different from baseline (* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$).
‡ Fourteen days after the feeding session had stopped.
§ *Togwa* value is significantly different from control value ($P < 0{\cdot}05$).

significantly at baseline (Table 2). In the stratified analysis for breast-feeding and sex (Table 5) we observed a significantly higher ($P < 0{\cdot}05$) proportion of children with isolated enteropathogens in the *togwa* group compared with the control group among the non-breast-feeding and male children at baseline. However, this difference did not change the trend of results observed in the unstratified analysis on the subsequent days except that, on the follow-up day, the proportion of female children who were isolated with enteropathogens in the *togwa* group did not differ significantly from baseline. There was also a significant reduction ($P < 0{\cdot}05$) from 28·6% at baseline to 5·3% on day 7 of those with enteropathogens in breast-feeding control children. However, on day 13 and follow-up day the proportions were not different from baseline. In non-breast-feeding control children those with enteropathogens increased slightly from 4·8% at baseline to 18·8% on day 7.

DISCUSSION

Effect of *togwa*

In this study, we have demonstrated a significant ($P < 0{\cdot}001$) decrease in the proportion of children with isolated faecal enteropathogens during the study period and on a follow-up day in association with consumption of *togwa* (Fig. 1). This observation supports our previous *in vitro* studies [14, 22, 23], which showed that *togwa* with pH $\leqslant$ 4 was able to suppress the growth of enteropathogenic bacteria. Similar findings from *in vitro* studies are also reported by Nout and colleagues [11] and Mensah and colleagues [12, 13]. A lower number of diarrhoea episodes among regular consumers of *togwa* was reported by Lorri and Svanberg [25] in a community based study. Brunser and colleagues [20] in Santiago reported a significantly lower incidence of diarrhoea, duration of episodes and carrier rates for enteropathogenic bacteria in young children fed on acidified milk for 6 months compared with children fed on nonacidified milk. Alm [16] and Lidbeck and colleagues [17] also reported that *Lactobacillus acidophilus* given as fermented milk resulted in decolonization of individuals with enteropathogens such as salmonella, *E. coli* and bacillus strains.

The inverse association of *togwa* consumption against the risk of colonization with enteropathogens was of a cause and effect relationship, seen over the study period and the effect increased with a higher intake frequency of *togwa* (Fig. 1). This may be attributed to three main mechanisms most likely acting in synergism. Firstly, the improved microbiological quality of *togwa* resulted in a lower transmission of enteropathogens through that particular food. Secondly, LAB present in the *togwa* may have survived the passage through the stomach [17, 27, 31] and exerted an inhibitory effect in the gastric canal through production of inhibitive factors [32, 33]. Thirdly, LAB colonized the intestines

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[17, 27, 31] resulting in competition for nutrients and space, thus precluding establishment of enteropathogenic bacteria in the intestines [34].

Effect of confounders

The strong association of *togwa* consumption against enteropathogenic colonization still persisted even after stratifying by all the potential confounders registered at baseline ruling out confounding effect by such variables. At baseline, however, the distribution of the socioeconomic characteristics of the households and the children themselves were similar except for sex and median age for stopping breast-feeding (Table 2). This partly explains the non-association of many of the confounders with enteropathogens at baseline, during the study period and on the follow-up day.

Stratifying the analysis by sex and breast-feeding, left the influence of *togwa* on the enteropathogenic faecal bacteria essentially unaltered (Table 5) indicating absence of confounding effect by these variables. The effect was still there even after stratifying the breast-feeding and non-breast-feeding children separately by age group of 6–12 months, 12–24 months and children older than 24 months (data not shown).

There is data that breast-feeding children are protected from diarrhoea infections [35, 36–38]. In this study we also observed that breast-feeding children in the control group were less likely to become colonized with enteropathogens during the study period (seen at day 7) compared with the non-breast-feeding children (Table 5). The freshly prepared (microbiologically safe) gruel which was provided during the study period seemed to have complemented the protective effect of breast-feeding against colonization with enteropathogens in the control children during the study period. This emphasizes WHO recommendation, that weaning children are given freshly prepared or adequately reheated foods [37, 39].

The prophylactic effect of *togwa* indicated in this study (see Table 4) is in coherence with that of Watkins and Miller [40]. They used gnotobiotic chicks as an animal model and observed that using LAB adjuncts protected the animals from getting colonized with enteropathogens. Lidbeck and colleagues [17], Johansson and colleagues [27] and Graham and colleagues [31] observed that LAB given as a lactic acid-fermented supplement to healthy volunteers resulted in an intestinal colonization with LAB that lasted up to 11 days after stopping the administration. These findings may offer an explanation to the extended effect on enteropathogenic inhibition observed in both *togwa* groups 14 days after feeding on *togwa* was stopped. The starter culture (*L. plantarum* 299v) used in preparing *togwa* 2 has been shown to survive the passage through the stomach as well as colonize the small intestine in human adults [27], and the same properties may therefore be present in the locally prepared *togwa* 1.

Type of enteropathogens

The higher frequency of campylobacter isolated in the asymptomatic children over the entire study period (Table 3) agrees with reports by Suan and colleagues [3] and Casalino and colleagues [4] who isolated campylobacter more frequently in asymptomatic children and salmonella, ETEC and shigella more frequently from children with diarrhoea. Čobeljič and colleagues [5] and Cravioto and colleagues [6] reported a higher frequency of ETEC and shigella in faeces from symptomatic children than from asymptomatic. These results support our findings of relatively few faecal swabs with ETEC and shigella since we dealt with non-diarrhoea children. In our previous aetiological study in Southern part of Tanzania (unpublished data), we found that 23% of healthy children were carriers of campylobacter and 19% carriers of either salmonella, shigella or ETEC.

EHEC:O157 was not isolated in any faecal swabs possibly because asymptomatic children normally do not carry this bacteria as part of their intestinal flora [7, 41]. The limitation of the agar, sorbitol-MacConkey we used can, however, not be overruled. Such agar has been reported to have a sensitivity of only 60% in detecting some serotypes of *E. coli*:O157 [42].

This study has demonstrated that regular consumption of *togwa*, with pH $\leqslant 4$, can protect young children from getting colonized with enteropathogenic bacteria. A regular intake of *togwa* once a day three times a week is sufficient to lower the risk of being colonized with enteropathogens. This may result in a lower risk of having diarrhoea later on. Use of strains known to survive the passage through the stomach and to colonize the intestines may have an additional advantage by extending the protective effect for some days in case of discontinued feeding.

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