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Influence of enteric bacteria, parasite infections and nutritional status on diarrhoea occurrence among 6-60 months old children admitted at a Regional Hospital in Morogoro, Tanzania

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Abstract: While nutritional, microbiological and immunological factors have been implicated in childhood diarrhoea in many countries, there is limited aetiological information in Morogoro Region of Tanzania. A case-control study was conducted to establish whether diarrhoea in 6-60 months old children admitted at a Regional Hospital in Morogoro, was attributable to enteric bacteria and/or parasites and the contribution of under-nutrition, as measured by weight-for-age below -2 SD. From January to September 2011, children admitted at the Hospital with (cases) and without diarrhoea (controls), were obtained by convenience sampling. Children's stool, weights, ages and information on socioeconomic, feeding, water and sanitation factors were obtained. Stool samples were analysed for *Escherichia coli* O157, *Shigella dysentriae, Campylobacter jejuni, Salmonella* species and enteric parasites. Logistic regression was used to identify their association with diarrhoea occurrence; and survival analysis used to assess associated risk, using associated hazard ratios (HR). Commonest bacteria isolated were *Salmonella*, more from controls, 45 (29.6%), than cases, 25 (16.6%); *S. dysentriae* and *C. jejuni* were only isolated from cases, while *E coli* O157 was not found. Enteric parasites were least prevalent; 4 (2.6%) for cases and 2 (1.3%) for controls. Although under-weight children had 38% increased risk of having diarrhoea than normal ones, this was not significant (HR = 0.98, p=0.928). Other factors found to significantly (p<0.05) influence diarrhoea occurrence included age when breastfeeding stopped, food(s) given, feeding utensils and the child´s toilet. In conclusion, childhood diarrhoea occurrence should warrant microbiological testing, for timely, appropriate treatment and prevention of transmission to others. Prevention and control measures for diarrhoea in children in Morogoro should include adequate breastfeeding, proper disposal of children's faeces and feeding children using cups rather than bottles. The increased occurrence of diarrhoea among malnourished children, particularly upon breast milk withdrawal, gives food and nutrition prominence among control measures.

Key words: children, diarrhoea, enteric bacteria, enteric parasites, nutritional status, Tanzania

Introduction

The commonest communicable diseases of man are not necessarily the best understood. For over 50 years, childhood diarrhoea has remained a serious problem in many countries, with the deaths from diarrhoea exceeding those from any other single cause. The global paediatric death

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toll due to diarrhoea exceeds that of Acquired Immunodeficiency Syndrome (AIDS), malaria and measles combined; and 37% of the diarrhoea cases occur in Sub-Saharan Africa (World Bank, 1999). Diarrhoea is more prevalent in developing countries due to lack of safe drinking water, poor sanitation, poorer health and nutritional status. Undernourished children are more likely to suffer from diarrhoea, and prolonged diarrhoea exacerbates poor health and malnutrition, creating a vicious cycle (UNICEF/WHO, 2009).

The prevalence of diarrhoea among under-fives in Tanzania is 15% (TDHS, 2010), with 23,900 annual deaths (UNICEF/WHO, 2009). In Morogoro region, the prevalence of diarrhoea among under-fives is 15.8% (TDHS, 2010), an increase from 12.1% in 2004 (TDHS, 2005). At the national level, 16% of under-fives have low weight-for-age, which reflects both chronic and acute under-nutrition. In Morogoro region, the prevalence of underweight (below -2 standard deviation units (SD) from the median of the WHO Child Growth Standards adopted in 2006) in this age group has been reported to be 19.1% (TDHS, 2010). Protein calorie malnutrition is the fifth most common cause of mortality (1.72%) in Morogoro region (Ngasongwa, 2007).

Nutritional, microbiological and immunological factors have been associated with diarrhoea (Long *et al*., 1999; Keusch *et al*., 2006). Diarrhoea has been reported to be a sign of gastrointestinal infections due to ingestion of bacteria, viruses or parasites spread by water, food, utensils and flies (UNICEF, 2009), giving aetiologic diagnosis high valuable for interventions and case management (Keusch *et al*., 2006). The bacteria implicated in childhood diarrhoea in developing countries are *Escherichia coli, Shigella, Campylobacter* and *Salmonella* (Motarjemi *et al*., 1993; UNICEF/WHO, 2009). With specific reference to Morogoro region, there is limited information on diarrhoea aetiology. An association has been reported between the syndrome and water (Ngasongwa, 2007), however, suggesting biological causes. Childhood under-weight status has also been found to be a risk factor for morbidity and mortality, in other developing countries, with 60.7% of deaths due to diarrhoea attributable to weight-for-age below -1 SD (Beau *et al*., 1987; Brown, 2003). This study carried out to establish whether diarrhoea in six to 60 month old children admitted at a Regional Hospital in Morogoro, Tanzania was attributable to enteric bacteria or parasites, as well as the contribution of underweight status of children.

Materials and methods

Study site

The study was conducted at Morogoro Regional Hospital, located in Morogoro Municipality (6^0,49'S; 37^0,40'E) in Tanzania. Morogoro Regional Hospital is a referral hospital for six districts namely: Morogoro Municipality, Morogoro Rural, Kilombero, Ulanga, Mvomero and Kilosa (Ngasongwa, 2007). The Paediatrics section of the hospital has three wards. The study involved children admitted in two of these wards: the ward for management of infectious diseases and the ward for management of non-infectious diseases. The third paediatric ward was excluded because it admits children under the age of one month.

Study design and sampling technique

A case-control study was used. A sample size of 303, comprising 151 cases and 152 controls was

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used to provide 90% power to detect a relative risk of 2.0, with 95% confidence interval; allowing for the possibility of a 30% proportion of controls having pathogens (Kirkwood & Sterne, 2003). The verbal informed consent of caretakers of 6-60 months old children was sought. Inclusion criterion for cases was admission at the Paediatric Infectious Diseases ward, and the caretaker's report of increase in the child's stool fluidity and frequency of passing of stool for at least two days. The children admitted at the ward for management of non-infectious diseases constituted the controls, excluding those who had had diarrhoea within the last two consecutive weeks. Cases and controls were obtained by convenience sampling (Mann, 2003). All children meeting case criteria and those meeting control criteria, admitted at the same time (Kirkwood & Sterne, 2003) of the same age group and residing in Morogoro region, were included in the study, for the period from January to September 2011.

Data collection

Weights of children were taken upon admission at the hospital. Data on socioeconomic factors, water and sanitation, feeding practices and occurrence of diarrhoea, was obtained from the caretakers by direct one-on-one interviews, using a structured questionnaire. A stool sample was collected once for each child, using labelled wide-mouthed, plastic containers with screw-top lids fitted with a long scoop (Carter & Lema, 2003). Part of the stool sample collected was transferred, using the scoop, into a labelled glass bijou bottle containing semisolid Cary Blair transport media, which inhibits Gram-positive bacteria and stops the multiplication of other bacteria (Cheesbrough, 2000). The samples, in both the plastic containers and bijou bottles were then transported, in a cold box, within one hour of collection, from the hospital to the Microbiology and Parasitology Laboratory, at Sokoine University of Agriculture, for analysis. Sample characteristics including colour, form and presence of blood or mucous were recorded.

Stool analysis for parasites

Stool samples were screened for enteric parasites by direct smear, involving mixing a small amount of stool with a drop of 0.85% physiological saline on a slide and examining microscopically. This was used to observe for helminth ova and larvae and trophozoites and cysts of *Giardia lambia* and *Entamoeba histolytica.* Modified Ziehl Nielsen staining was used for identification of *Cryptosporidium* oocysts (Carter & Lema, 2003).

Stool analysis for bacteria

In this study, the focus was isolation of four specific bacteria namely: *Salmonella*, *Shigella*, *Campylobacter* and *Escherichia coli* O157. The main media used included MacConkey agar (HI Media, Mumbai), Sorbital MacConkey agar (Oxoid, Basingstoke), Blood agar (HI Media, Mumbai), Xylose Lysine Deoxycholate agar (Accumix: Tulip group, Mumbai), Salmonella Shigella agar (HI Media, Mumbai), Charcoal cefoperazone deoxycholate agar (Oxoid, Basingstoke). Other media used included the Wellcolex *E. coli* O157kit (Remel (Europe) Limited, Dartford Kent), Mannitol Selenite Faecal broth (Oxoid, Basingstoke), Triple Sugar Iron agar (HI Media, Mumbai), Urea agar (DIFCO, Michigan), Cary-Blair (Oxoid, Basingstoke) and the sugars glucose, lactose, sucrose, mannitol and citrate (Sigma, Missouri).

The samples were inoculated on MacConkey agar and Blood agar and incubated at 37°C

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for 24 hours, to obtain bacteria of the genus *Escherichia.* The positive colonies were then sub-cultured on Sorbitol MacConkey agar (SMAC), containing cefixime and tellurite, and incubated at 37°C for 24 hours. Culturing on Triple sugar iron (TSI) agar was also done, to screen for characteristic *E. coli* fermentation of lactose, glucose and sucrose (WHO, 1995a; Evaluations & Standards Laboratory, 2010). Suspected bacteria colonies were subjected to a latex agglutination test. *E. coli*O157 was identified by its agglutination in *E. coli* O157 antiserum (WHO, 1995a; Bridson, 1998).

Charcoal cefoperazone deoxycholate agar, a *Campylobacter* blood-free selective medium, (modified CCDA-preston), with addition of the modified Bolton broth selective growth supplement SR208E, were used to grow *Campylobacter jejuni* (Bridson, 1998; Pumbwe & Piddock, 2004; Granato *et al*. 2010). To the agar, antibiotics namely celexin, rifampicin and nystatin were also added, to inhibit other Gram-negative bacteria, Gram-positive bacteria and fungal growth respectively (Pumbwe & Piddock, 2004). Incubation on the modified CCDA was done at 37°C for 48 hours, in an anaerobic candle jar (Linton *et al*., 1997; Bridson, 1998; Evaluations & Standards Laboratory, 2007a); after which a loopful of the suspected colonies isolated on the agar were subjected to the hippurate test (Nakari *et al*., 2008). *C. jejuni* was identified by its ability to hydrolyze *N*-benzoylglycine (hippurate) to benzoic acid and glycine (Denis *et al*., 1999; Steele *et al*., 2002; Nakari *et al*., 2008; Khanzadi *et al*. 2010). Gram staining of the suspected colonies was also done, to observe the characteristic spiral or S-shape, Gram-negative appearance of *C. jejuni* (Carter & Lema, 2003; Granato *et al*. 2010).

Stool samples were inoculated on Xylose lysine deoxycholate (XLD) agar, for moderate to high selectivity of *Shigella*, as well as MacConkey agar, and incubated at 37°C for 24 hours (Bridson, 1998). Suspected bacteria colonies were isolated and Gram-stained, to observe the characteristic small Gram-negative rod-shape morphology of *Shigella* species (Evaluations & Standards Laboratory, 2007b). The positive colonies were subjected to biochemical tests including inoculation on TSI agar, (Bridson, 1998) as well as in the sugars: sucrose, mannitol, glucose, lactose and citrate (WHO, 1995a). *Shigella dysentriae* was distinguished by its inability to ferment mannitol up to 48 hours of incubation at 37°C (Cheesbrough, 2000; Evaluations & Standards Laboratory, 2007b).

For *Salmonella* species, samples were enriched by inoculation in Mannitol selenite faecal broth, at 37°C for 18 hours. They were then cultured on XLD agar, Salmonella-Shigella agar, Blood agar and MacConkey agar at 37°C for 24 hours. Suspected bacteria colonies on XLD agar were cultured for a further 24 hours (Bridson, 1998), for production of hydrogen sulphide gas (Hendriksen, 2003). The colonies were then isolated and Gram-stained for observation of the characteristic Gram negative rod-shape morphology of *Salmonella*. Positive colonies were inoculated on TSI agar, urea agar as well as in the sugars: sucrose, mannitol, glucose, lactose and citrate (Bridson, 1998; Cheesbrough, 2000; Hendriksen, 2003). *S. typhi* was distinguished by its inability to produce gas in TSI agar, *S. paratyphi* by its inability to produce hydrogen sulphide in TSI agar and non-typhoidal *Salmonella* by their ability to ferment citrate (Bridson, 1998; Cheesbrough, 2000).

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Data analysis

The data were analyzed using Epi info, version 3.4.3, SPSS, version 16.0 and ENA for SMART. Weight-for-age z-scores (WAZ) were obtained and classified into three categories: > -2 SD, -2 to -3 SD and < -3 SD, for normal, moderately underweight and severely underweight, respectively, relative to the National Center for Health Statistics/World Health Organization (NCHS/WHO) reference median weight (Hamill, 1977; WHO, 1978). Nutritional status, presence of enteric bacteria or parasites and other factors were compared between cases and controls, and odds ratio (OR) used to assess their influence (Edwardes, 2001; Mann, 2003; Bloss *et al*., 2004) on diarrhoea occurrence. Linear regression was used to find the association between diarrhoea occurrence and the age of children. Stepwise logistic regression was used to assess the influence of socioeconomic and other factors on the occurrence of diarrhoea. A survival analysis approach was used to identify factors influencing diarrhoea occurrence by age (Essebag *et al*., 2005; Hsu, 2006; Langholz, 2010). For survival analysis, semi-parametric Cox proportional hazard models (Woodward, 2005) were used, survival time defined as age of children in months. Significance tests about subsets of parameters were derived by comparison of hazard ratios (HR) and -2 log likelihoods, according to likelihood ratio test chi-square distribution (Cox, 1972).

Ethical consideration

The Medical Research Coordination Committee, of the National Institute for Medical Research (NIMR), Dar es Salaam, Tanzania, granted the ethical clearance, reference number: NIMR/HQ/R. 8a/Vol. IX/1023, for conducting this study. A verbal informed consent was sought from caretakers of each of the 6-60 months old children admitted during the study period. Each caretaker of the admitted children was met and a thorough explanation of the study was given, with assurance of provision of stool analysis results. The children included in this study were those whose caretakers understood the study, accepted to participate fully and provided a means to be contacted at any time to give them the stool analysis results for their children.

Results

Descriptive statistics

Of the 303 children in the study, majority resided in Morogoro Municipality 243 (80.2%), many lived in households comprising five or more people 139 (45.9%), and their mothers were housewives 143 (47.2%), between the ages of 20 and 30 years 154 (50.8%), with primary school education attained 205 (67.7%). Slightly over half of the children 173 (57.1%) lived in households with a water tap and a latrine 169 (55.8%). Although many were from households that boiled drinking water 156 (51.5%), there were also many 129 (42.6%) from households that drank water that was neither boiled nor treated, and some of the mothers 48 (15.8%) usually had the children defecate on the ground, for them to dispose-off later.

Most of the children in the study were two years old or younger 254 (83.8%), with those six months to one year old constituting nearly half, 146 (48.2%). Only 64 (21.1%) children had been exclusively breastfed for six months. There were more cases 116 (76.8%) than controls 106 (69.7%) among children who had started drinking water before the age of six months and fewer cases 3 (2%) than controls 20 (13.2%) among the children who stopped breastfeeding at two

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Table 1: Effect of the enteric pathogens isolated on diarrhoea occurrence

Bacteria/ Parasite	Pathogen	Diarrhoeal (%) - N=151	Control group (%) - N=152	OR	95% CI	P-value
Bacteria	*Salmonella typhi*	4 (2.6)	14 (9.2)	0.27	0.07 – 0.90	0.016*
	Salmonella paratyphi	17 (11.3)	19 (12.5)	0.89	0.42 – 1.88	0.739
	Non-typhoidal *Salmonella*	4 (2.6)	12 (7.9)	0.32	0.08 – 1.09	0.042*
	Shigella dysentriae	3 (2.2)	0 (0.0)			
	Campylobacter jejuni	5 (3.3)	0 (0.0)			
	Escherichia coli O157	0 (0.0)	0 (0.0)			
Parasite	*Hook worm* ova	0 (0.0)	1 (0.7)	0.00	0.00 – 17.51	0.502
	Giardia lambia cysts	1 (0.7)	1 (0.7)	1.01	0.00 – 37.16	0.749
	Cryptosporidium oocysts	1 (0.7)	0 (0.0)			
	Tape worm ova	1 (0.7)	0 (0.0)			
	Entamoeba hystolytica Trophozoites	1 (0.7)	0 (0.0)			

***Significant at P < 0.05**

years or older. Diarrhoea occurrence also varied among the children with diarrhoea and the control group by the feeding utensils of the children. Among children fed using bottles there were more cases 82 (54.3%) than controls 30 (19.7%); while among the children fed using cups, there were fewer cases 72 (47.7%) than controls 123 (80.9%). The drinks most commonly given included water 303 (100%) and juice 303 (100%) while the food(s) commonly eaten included porridge 263 (86.8%), vegetables 236 (77.9%) and stiff maize-porridge ("ugali") 176 (58.1%). There were fewer diarrhoea cases 12 (7.9%) than controls 28 (18.4%) among children not fed porridge, and more cases 51 (33.8%) than controls 16 (10.5%) among those not fed vegetables.

Table 2: Influence of socioeconomic and other factors on diarrhoea occurrence

Factor	OR	95% CI	β (SE)	Wald	P-value
Age of the child			0.063 (0.014)	21.328	0.000*
Age of the caretaker			0.022 (0.017)	1.584	0.208
Sex of the child (M/F)	1.16	0.72 – 1.89	0.152 (0.233)	0.423	0.515
Birth order (1st/others)	0.78	0.47 – 1.28	2.639 (1.232)	7.788	0.168
Child plays outside the house (Yes/No)	0.93	0.55 – 1.57	-0.073 (0.253)	0.084	0.772
Age complementary feeding started (≥6/<6)	0.51	0.27 – 0.97	0.437 (0.635)	9.065	0.337
Age breastfeeding was stopped (≥24/<24)	0.13	0.03 – 0.49	-1.451 (1.163)	22.47	0.000*
Drinks given					
Water (boiled/unboiled)	0.77	0.44 – 1.34	-0.003 (0.282)	3.761	0.584
Milk (Yes/No)	0.68	0.42 – 1.12	0.219 (0.247)	2.131	0.831
Home-made fruit juice (Yes/No)	0.77	0.47 – 1.28	-0.255 (0.243)	1.101	0.294
Processed drinks (Yes/No)	0.69	0.38 – 1.25	-0.288 (0.292)	0.971	0.324

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Foods given					
Cereal-based porridge (Yes/No)	13.51	5.66 – 32.84	0.357 (0.450)	8.882	0.261
Fruits (Yes/No)	1.53	0.88 – 2.66	0.426 (0.265)	2.577	0.108
Vegetables (Yes/No)	0.23	0.12 – 0.44	-20.942 (0.00)	21.73	0.000*
Eggs (Yes/No)	1.28	0.62 – 2.68	0.249 (0.349)	0.510	0.475
Fish (Yes/No)	1.66	0.95 – 2.90	0.505 (0.369)	3.534	0.060
Feeding utensils					
Bottle (Yes/No)	4.83	2.81 – 8.34	0.924 (0.345)	7.164	0.007*
Cup (Yes/No)	0.21	0.12 – 0.37	-0.861 (0.349)	6.092	0.014*
Spoon (Yes/No)	11.29	6.37 – 20.11	0.432 (0.311)	1.960	0.162
Hands (Yes/No)	0.86	0.54 – 1.39	0.156 (0.321)	0.237	0.627
Livelihood of the caretaker				15.30	0.004*
Salary (Yes/No)	0.29	0.11 – 0.74	-0.56 (0.322)	3.022	0.082
Small business (Yes/No)	0.84	0.45 – 1.58	0.179 (0.361)	0.246	0.62
Farmer (Yes/No)	0.65	0.37 – 1.15	-0.842 (1.255)	0.45	0.502
Housewife (Yes/No)	2.21	1.36 – 3.59	0.997 (0.513)	3.78	0.052
Education of the caretaker	0.72	0.39 – 1.32	-1.174 (0.671)	3.385	0.496
Household size			0.649 (0.567)	5.943	0.430
Household water source (Own tap/others)	0.89	0.55 – 1.44	0.916 (1.643)	1.615	0.978
Drinking water preparation (Boiling/Not)	0.82	0.51 – 1.32	-0.041 (0.512)	0.669	0.880
Drinking water store	1.82	0.79 – 4.26	1.240 (0.802)	3.100	0.212
Household toilet (Flushing toilets/latrines)	0.96	0.59 – 1.55	0.042 (0.231)	0.032	0.857
Child's toilet				17.94	0.003*
Toilet or latrine (Yes/No)	0.14	0.03 - 0.52	-2.89 (1.197)	5.829	0.016*
Potty (Yes/No)	0.72	0.44 – 1.16	-2.303 (1.121)	0.004	0.952
Diapers or cloth (Yes/No)	1.66	0.99 – 2.80	-1.604 (1.093)	2.152	0.142
The ground (Yes/No)	1.85	0.94 – 3.65	0.08 (1.32)	4.223	0.040*

***Significant at P < 0.05**

Enteric pathogens isolated

The commonest bacteria isolated were *Salmonella* species (Table 1), being isolated from more children admitted without diarrhoea (controls) 45 (29.6%) than those admitted with diarrhoea (cases) 25 (16.6%). *Shigella dysentriae* and *Campylobacter jejuni* were only isolated from cases. Enteric parasites were least prevalent; 4 (2.6%) for cases and 2 (1.3%) for controls. All samples were negative for *Escherichia coli* O157. *Campylobacter jejuni* positive stools were watery, while *Shigella dysentriae* positive stools were mucoid. *Salmonella* positive stools were mostly semi-solid, but there were instances where they were bloody, mucoid or solid. *Salmonella typhi* positive stools were either mucoid, 4 (22.2%), semi-solid, 8 (44.4%) or solid 6 (33.3%); *Salmonella paratyphi* stools were either bloody, 1 (2.8%), mucoid, 12 (33.3), semi-solid, 18 (50%), or solid, 5 (13.9%); while non-typhoidal *Salmonella* stools were either bloody, 1 (6.3), mucoid, 3 (18.8%), semi-solid, 8 (50%), or solid 4 (25%).

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Effect of nutritional status and other factors on diarrhoea occurrence

The prevalence of low weight-for-age (WAZ < -2 SD) was 29.2% among children with diarrhoea and 23.1% among the children without diarrhoea. The underweight children had 38% increased risk of having diarrhoea than the normal children did, but nutritional status showed low influence (OR = 1.38, P = 0.427) and low risk (HR = 0.98, P = 0.928) for diarrhoea occurrence at any age of the children (Table 3).

Diarrhoea occurrence was influenced by age of the child, age at which breastfeeding had been stopped, foods given, feeding utensils and the child´s toilet/disposal of the child's faeces (Table 2). The results of linear regression showed that there was significant correlation (P = 0.000) between age and diarrhoea occurrence, with age being responsible for 8% of occurrence (correlation coefficient (r^2) = 0.08). Children who stopped breastfeeding at two years or later had significantly reduced risk for diarrhoea occurrence (OR = 0.13, P = 0.000, β = -1.451) and so did those who had been fed vegetables (OR = 0.23, P = 0.000, β = -20.942). Bottle-fed children had significantly increased risk (OR = 4.83, P = 0.007, β = 0.924), while cup-fed children had reduced (OR = 0.21, P = 0.014, β = -0.861). Where children used the household toilet, there was significantly reduced risk for diarrhoea (OR = 0.14, P = 0.016), whereas children allowed to defecate in the compound or on the floor in the house had increased risk (OR = 1.85, P = 0.04).

The age at which breast feeding was stopped (age weaned), food(s) given and children playing outside the house showed increased risk for diarrhoea occurrence at any given age (Table 3). Bacterial infections also increased risk for diarrhoea occurrence by age, particularly infection with *Salmonella typhi* and *Campylobacter jejuni.*

Table 3: Risk for diarrhoea occurrence by various factors at any given age of the child

Factor	Response	Hazard Ratio	95% C. I.	β	S. E.	P-Value
Bacteria	*Salmonella* (non-typhoidal/None)	0.382	0.141 – 1.035	-0.961	0.508	0.059
	Salmonella (*paratyphi*/None)	0.828	0.499 – 1.374	-0.189	0.259	0.465
	Salmonella (*typhi*/None)	0.283	0.104 – 0.766	-1.263	0.509	0.013
	Campylobacter (None/*jejuni*)	0.356	0.157 – 0.811	-1.032	0.42	0.014
	Shigella (None/*dysentriae*)	0.414	0.131 – 1.302	-0.883	0.585	0.131
Nutritional status	Underweight/Normal	0.975	0.565 – 1.683	-0.025	0.278	0.928
Age weaned (months)	> 24 /< 12	0.811	0.100 – 6.599	-0.21	1.07	0.845
	12 - 24/< 12	0.955	0.367 – 2.484	-0.046	0.488	0.925
	Not yet/< 12	4.081	1.730 – 9.626	1.406	0.438	0.001*
Feeding utensils	Bottle (Yes/No)	2.256	1.356 – 3.752	0.813	0.26	0.002*
	Bowl (Yes/No)	2.219	1.003 – 4.911	0.797	0.405	0.049*
	Cup (Yes/No)	0.366	0.222 – 0.604	-1.005	0.256	0.000*
	Hands (Yes/No)	0.418	0.250 – 0.697	-0.874	0.261	0.001*
	Spoon (Yes/No)	2.913	1.620 – 5.238	1.069	0.299	0.000*

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Child plays outside	Yes/No	0.546	0.302 – 0.984	-0.606	0.301	0.044*
Child fed porridge	Maize/Maize-soya blend	0.676	0.344 – 1.327	-0.392	0.344	0.255
	Many/Maize-soya blend	0.659	0.316 – 1.375	-0.417	0.375	0.267
	Millet/Maize-soya blend	0.571	0.267 – 1.223	-0.56	0.389	0.149
	None/Maize-soya blend	0.171	0.049 – 0.603	-1.765	0.642	0.006*
Child fed rice	Yes/No	0.34	0.166 – 0.693	-1.08	0.364	0.003*
Child fed vegetables	Beans and Greens/Beans	0.442	0.188 – 1.041	-0.816	0.437	0.062
	Greens/Beans	0.732	0.377 – 1.422	-0.312	0.339	0.357
	None/Beans	3.449	1.861 – 6.391	1.238	0.315	0.000*
Child´s drinking water	Boiled and Bottled/Boiled	0.536	0.220 – 1.305	-0.624	0.454	0.169
	Bottled/Boiled	0.489	0.249 – 0.957	-0.716	0.343	0.037*
	Unboiled/Boiled	0.624	0.340 – 1.146	-0.472	0.31	0.128

***Significant at P < 0.05**

Discussion

A hospital-based case-control design was used to determine the importance of specific enteric bacteria in diarrhoea occurrence in young children, as well as the possible contribution of their nutritional status. The case-control design was chosen to enable evaluation of many risk factors (Mann, 2003); and hospital controls, being ill, would have relatively better exposure recall than population-based controls (Shulz & Grimes, 2002). However, the case control design would make it difficult to establish the timeline of the pathogens and nutritional status exposing children to diarrhoea occurrence (Rose & Van der Laan, 2009).

Low weight-for-age (under-weight) indicates a history of poor health or nutritional insult; including recurrent illness and/or starvation (Bloss *et al*., 2004). Although it does not distinguish between wasting and stunting, it represents a combination of both aspects and has a high positive predictive value as an indicator for child malnutrition in developing countries (WHO, 1995b). The prevalence of under-weight among children in this study was 34%, higher than the 19.1% Morogoro Regional average given in the Tanzania Demographic and Health Survey (2010) report (TDHS, 2010). This may be attributed to the fact that the children, being ill, may have been more likely to have their nutritional status compromised; as was also found in a similar hospital-based study in Senegal (Beau *et al*., 1987).

Anthropometric indicators of poor nutritional status have been noted to affect not just the incidence (Victora *et al*., 1992; Fishman *et al*., 2004), but both duration (Black *et al*., 1984) and severity of diarrhoea as well (Brown, 2003). The underweight children in this study had 38% increased risk of having diarrhoea than the normal children. However, although low weight-for-age was more prevalent in children with diarrhoea in this study, this was not statistically significant. A case-control study in Bangladesh, with community-controls, also found a high

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prevalence of underweight in both children with diarrhoea and those without (Albert *et al*., 1999). In Brazil, however, even after adjusting for weight loss due to dehydration, low weight-for-age still showed a strong effect on diarrhoea occurrence and associated mortality (Victora *et al*., 1992). The nutritional status of children can be undermined by feeding them energy-dense, low-protein foods. In a study in Kenya, it was found that many women were the family's primary labourers, and unable to regularly breastfeed; they gave water and porridge to their children soon after birth (Bloss *et al*., 2004). Among Guatemalan children, malnutrition attributed to poor weaning diets, predisposed children to diarrhoea by lowering resistance to infection, increasing the risk of disability and death (Scrimshaw *et al*., 1968). These findings suggest that the effect of under-weight on diarrhoea occurrence in children may be influenced by other factors, which may vary by place and/or the children themselves.

The commonest bacteria isolated were *Salmonella* species, being isolated from more controls than cases. *Shigella dysentriae* and *Campylobacter jejuni*, although less isolated, were only isolated from cases. *Campylobacter* infection was characterised by watery stools, *Shigella* infection by mucoid stools while *Salmonella* infection(s) by either bloody, mucoid, semi solid or solid stools. Watery diarrhoea has also been found to be the commonest symptom of *Campylobacteriosis* in North India (Coker *et al*., 2002). In other countries, however, there has been different manifestation: bloody stools with mucous and/or pus in Turkey and Pakistan (Ali *et al*., 2003). *Salmonella* have been associated with both diarrhoea and constipation (Stevenson, 1987; Simango & Mbewe, 2000; WHO, 2011). Enteric bacteria have been associated with diarrhoea in other similar settings; some also found among healthy people (Stevenson, 1987). The findings of this study show that change in frequency and fluidity of children´s stool, especially with mucous or blood, suggests enteric pathogen infection.

Other factors found to significantly influence diarrhoea occurrence by age included age the child was weaned, the food(s) given, the child´s toilet and whether or not the child had started playing outside the house. Children weaned at two years or later had reduced risk for diarrhoea occurrence at any age. Bottle-fed children had increased risk while cup-fed children had reduced, and spoon-fed children had increased while hand-fed children had reduced risk. Where children were reported to be using toilets, latrines or potties there was also reduced risk. These results suggest the protective role of breast feeding against diarrhoea, and increased risk where there was poor excreta disposal, suggesting that the diarrhoea may have been of infectious origin. There is also an indication of diarrhoea occurrence being associated with hygiene practices of caretakers; use of feeding utensils that are more difficult to clean, such as bottles, increase risk for diarrhoea occurrence. Such factors have been associated with enteric pathogen transmission in developing countries (Kotloff *et al*., 1999; Coker *et al*., 2002).

In conclusion, the change in frequency and fluidity of children´s stool, especially with mucous or blood, should warrant microbiological testing, for timely, appropriate treatment and prevention of transmission to others. Diarrhoea may be prevented by adequate breastfeeding and good sanitation and hygiene practices including proper excreta disposal and adequate cleaning of feeding-utensils, and health education of both mothers and their children. Increased occurrence of diarrhoea among malnourished infants, and particularly during and after weaning, gives food and nutrition prominence among the control measures. With both malnutrition and the exposure to enteric infection(s) as major public health problems, among

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young children, successful control may lie heavily upon efforts directed equally against both.

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Conflict of interest

Neither the authors nor the sponsor of this study has had any financial or other affiliations that could compromise the content of this manuscript.

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