ACTA PÆDIATRICA
NURTURING THE CHILD

Acta Pædiatrica ISSN 0803-5253

REGULAR ARTICLE

Presence of *Giardia lamblia* in stools of six- to 18-month old asymptomatic Malawians is associated with children's growth failure

Kirsi-Maarit Lehto (kirsi-maarit.lehto@tuni.fi)[1], Yue-Mei Fan[1], Sami Oikarinen[2], Noora Nurminen[2], Lotta Hallamaa[1], Rosa Juuti[3], Charles Mangani[1,4], Kenneth Maleta[4], Heikki Hyöty[2,5], Per Ashorn[1,6]

1.Center for Child Health Research, Faculty of Medicine and Health Technology and Tampere University Hospital, Tampere University, Tampere, Finland
2.Faculty of Medicine and Health Technology, Virology, Tampere University, Tampere, Finland
3.EPID Research Oy, Espoo, Finland
4.School of Public Health and Family Medicine, University of Malawi, Blantyre, Malawi
5.Fimlab Laboratories, Tampere, Finland
6.Department of Pediatrics, Tampere University Hospital, Tampere, Finland

Keywords
Asymptomatic, Children, Growth, Nutrient supplements, Pathogens

Correspondence
Dr. Kirsi-Maarit Lehto, Center for Child Health Research, Faculty of Medicine and Health Technology, Tampere University, FI-33014 Tampere, Finland.
Tel: +358 504201494 |
Fax: +358 32134473 |
Email: kirsi-maarit.lehto@tuni.fi

Received
28 November 2018; revised 27 March 2019; accepted 26 April 2019.

DOI:10.1111/apa.14832

ABSTRACT

Aim: Despite high pathogen burden and malnutrition in low-income settings, knowledge on relationship between asymptomatic viral or parasitic infections, nutrition and growth is insufficient. We studied these relationships in a cohort of six-month-old Malawian infants.

Methods: As part of a nutrient supplementation trial for 12 months, we documented disease symptoms of 840 participant daily and anthropometric measurements every three months. Stool specimens were collected every six months and analysed for *Giardia lamblia*, *Cryptosporidium* species and enterovirus, rotavirus, norovirus, parechovirus and rhinovirus using polymerase chain reaction (PCR). The prevalence of the microbes was compared to the children's linear growth and the dietary.

Results: The prevalence of the microbes was similar in every intervention group. All age groups combined, children negative for *G. lamblia* had a mean standard deviation (SD) of −0.01 (0.49) change in length-for-age *Z*-score (LAZ), compared to −0.12 (0.045) among *G. lamblia* positive children (difference −0.10, 95% CI −0.21 to −0.00, p = 0.047). The LAZ change difference was also statistically significant (p = 0.042) at age of 18–21 months but not at the other time points.

Conclusion: Asymptomatic *G. lamblia* infection was mainly associated with growth reduction in certain three-month periods. The result refers to the chronic nature of *G. lamblia* infection.

INTRODUCTION

Globally, linear growth failure or stunting affected an estimated 154.8 million, 22.9% of under five-year-old children in 2016 (1). Inadequate nutrition and intestinal infections are considered to be the main causes of childhood stunting (2) but results from antibiotic and probiotic interventions have been insufficient (3). In addition, Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) study did not find diarrhoea to be a major risk factor for poor growth. However, the study identified asymptomatic enteropathogen burden as an important contributor to growth faltering in children (4).

Giardia lamblia, also called as *Giardia duodenalis* or *Giardia intestinalis*, and *Cryptosporidium* species are environmentally ubiquitous protozoa and the most common enteric parasites infecting humans worldwide (5). In low-income countries, asymptomatic infections are very common, with prevalence values of, for example, giardiasis in paediatric populations being typically around 30% (6). The

Abbreviations
CI, Confidence intervals; LAZ, Length-for-age *Z*-score; LNS, Lipid-based nutrient supplementation; PCR, Real-time polymerase chain reaction; SD, Standard deviation; WHO, World Health Organization; WLZ, Weight-for-length *Z*-score.

Key notes

- Few studies have addressed the association of asymptomatic viral or parasitic infections and growth.
- We studied the epidemiology of two parasites and five viruses among asymptomatic Malawian children at six to 18 months of age and observed that primarily the asymptomatic *Giardia lamblia* infection was associated with subsequent growth reduction.
- To better assess the growth impact of non-chronic viral infections, more sensitive outcome variables are recommended with short follow-up periods.

prevalence of enteric pathogens or their consequences without diarrhoea, particularly in young children, is still poorly understood (7). However, it has been suggested that acute infections impact the children's linear growth through a direct as enteric and indirect as respiratory pathways (8).

A primary aim of this study was to test whether the prevalence of *G. lamblia*, *Cryptosporidium* species, enterovirus, rotavirus, norovirus, parechovirus and rhinovirus, detected in faecal samples among asymptomatic Malawian children at age of six, 12 and 18 months, is associated with linear growth reduction in the subsequent three-month period. A secondary aim was to study if a dietary supplementation would have an impact on the prevalence of the studied microbes in the children's stools.

SUBJECTS AND METHODS

Study design and specimen collection

This was a prospective cohort study, nested in a clinical Lungwena Child Nutrition Intervention Study (LCNI-5) registration ID: NCT00524446, assessing the effect of dietary supplementation with lipid-based nutrient supplements (LNS) on early childhood growth (9). The trial was conducted in Lungwena and Malindi, in two rural Malawian communities. Potentially eligible participants were identified through community census in the study area and invited to an enrolment session, where infants were screened for eligibility. The inclusion criteria included age 5.50–6.50 months, residence in the study area and informed consent from an authorised guardian. The exclusion criteria were weight-for-length *Z*-score (WLZ) <80% of the World Health Organization (WHO) reference median or presence of oedema, severe illness warranting hospitalisation on the enrolment day, history of peanut allergy, concurrent participation in another clinical trial and any symptoms of food intolerance within 30 minutes after ingesting a five *g* test dose of LNS used in the trial (9).

The study population was enrolled between 28 January 2008 and 25 May 2010 and comprised of 840 six-month-old infants. After the enrolment, the participants were provided with one of the four dietary for 12 months. Dietary supplementation included either 54 g/day of milk or soy containing LNS, 72 g/day fortified maize–soy flour or in a control group, no extra food supplements.

The morbidity information was used to exclude those participants having illness symptoms at collection time. We collected data with picture calendar recorded by guardian. The calendar had separate columns for the following symptoms: fever, cough, diarrhoea and other. The recorded information was reviewed and cross-checked by fieldworkers at each two weekly food delivery visit for completeness. From the information we collected at enrolment, 31 children had had diarrhoea within 14 days prior to stool sample collection, 22 within seven days and 11 within two days. This information describes the data reported before the first (inclusion) sample was taken. Those participants who did not have the symptoms and were healthy in their guardians' views comprised our study subjects.

Faecal samples were collected at child age of six, 12 and 18 months to detect the prevalence of the pathogens. Guardians collected a faecal sample from the participants at each time point and brought the sample in a 30 mL stool container to the health centre. The time between defecation and faecal freezing was not more than six hours. Bacterial findings (10,11) and prevalence of selected viruses and parasites and their predictors (12) in Malawian children as part of the same nutritional trial have been reported earlier. Now, we report viral and parasitic results in relation to children's growth and the nutrition interventions.

The trial adhered to the principles of the Declaration of Helsinki and regulatory guidelines in Malawi. The research and ethics committee of the College of Medicine, University of Malawi and the ethical committee of the Pirkanmaa Hospital District, Finland reviewed and approved the trial protocol.

Sample preparations and real-time polymerase chain reaction (PCR) analysis

We isolated viruses and parasites using nucleic acids QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) from 10% stool suspension. We used real-time PCR to characterise enterovirus, rotavirus, norovirus, parechovirus and rhinovirus (13,14), and parasites *G. lamblia* and *Cryptosporidium* species (15). We considered a specimen as a positive sample if two or three of the triplicate PCR runs gave a positive test result. We excluded the data if the actual date of the stool sample was off by plus or minus four weeks from the prescheduled target date.

Anthropometric indices

Trained research assistants took anthropometric measurements at every three months from the child age of six to 21 months. Assistants measured length using a Kiddimetre length board (Raven Equipment Ltd., Essex, UK). They weighed the children using a SECA 735 electronic child weighing scale (Chasmors Ltd., London, UK). We calculated length-for-age *Z*-scores (LAZ) and WLZ using WHO Child Growth Standards (STATA igrowup package) (16). We excluded measurement if the actual measurement date was off by plus or minus four weeks from the target age.

Statistical analysis

For prevalence of viruses and parasites, we calculated percentages by intervention group as number of positive samples at each study visit. For LAZ and change in LAZ, we calculated group means and used least squares regression to estimate differences between groups. For all time points combined, we took intragroup correlation due to multiple measurements per participant into account by using robust standard errors for clustered data. We rejected a null hypothesis if two-sided $p < 0.05$. Prevalence of virus and parasite by intervention group is presented without covariate adjustment. Analysis of association between virus or parasite and LAZ and change in LAZ is adjusted for child's virus or parasite status at enrolment, maternal height, maternal body mass index at enrolment, child sex, maternal

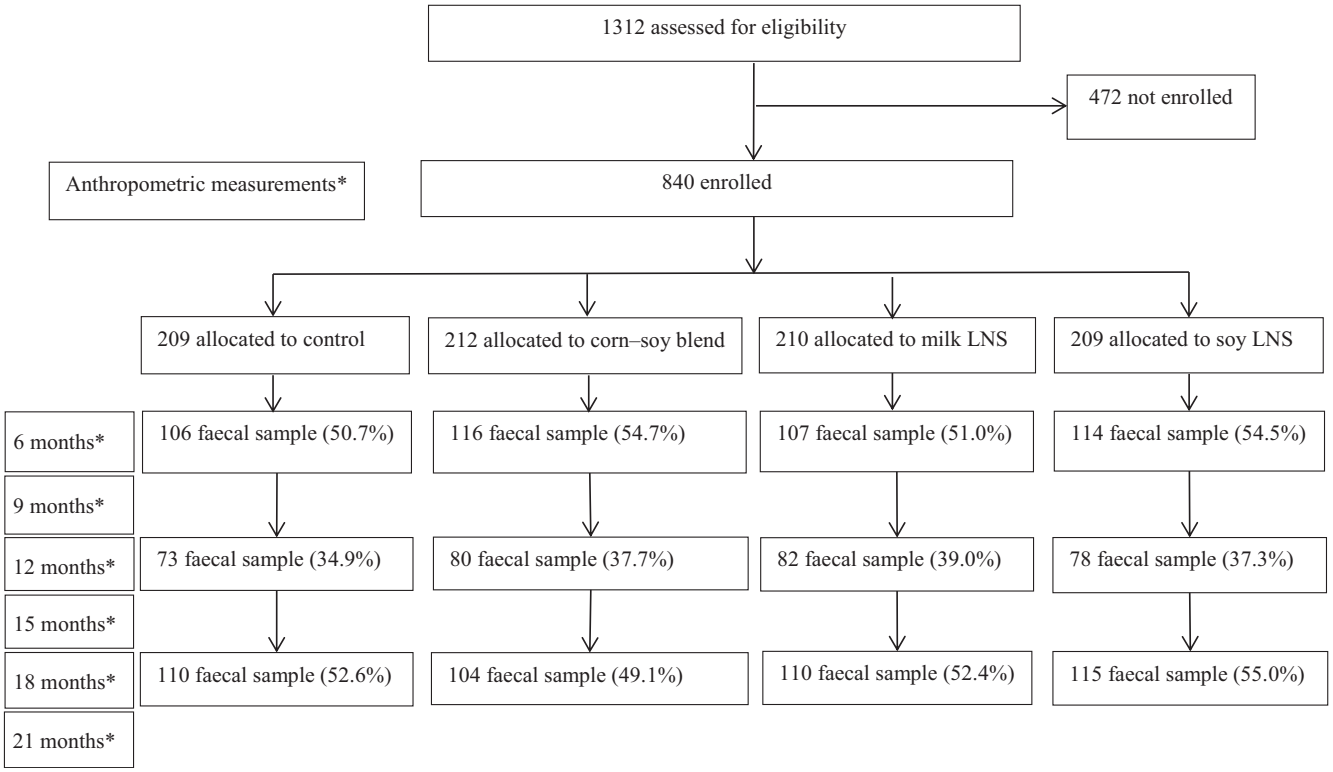

Figure 1 Participant flow.

Table 1 Baseline characteristics of participants at enrolment by intervention group

	Intervention group			
Participant characteristics	Control	Corn-soy blend	Milk LNS	Soy LNS
Number of participants	175	175	175	181
Infant male sex N (%)	95 (54.3%)	85 (48.6%)	90 (51.4%)	86 (47.5%)
Mean (SD) age, months	6.0 (0.2)	6.0 (0.3)	6.0 (0.2)	6.0 (0.2)
Length, cm	63.0 (2.1)	62.9 (2.2)	62.7 (2.1)	62.7 (2.1)
LAZ	−1.73 (0.97)	−1.68 (0.97)	−1.79 (0.99)	−1.80 (0.96)
WLZ	0.39 (0.99)	0.49 (1.07)	0.55 (1.03)	0.41 (1.11)
Socio-economic score[†]	−0.01 (1.04)	0.06 (1.05)	−0.02 (0.99)	0.00 (0.92)
Maternal education, years	3.4 (3.2)	4.0 (3.5)	3.0 (3.1)	3.6 (3.1)
Sanitary facilities N (%)				
None	13 (7.4%)	6.9%)	12 (6.9%)	11 (6.1%)
Vent. impr. pit latrine	1 (0.6%)	2 (1.1%)	0 (0.0%)	0 (0.0%)
Regular pit latrine	157 (89.7%)	157 (89.7%)	161 (92.0%)	166 (1.7%)
Other/missing	4 (2.3%)	4 (2.3%)	2 (1.1%)	4 (2.2%)
Water source N (%)				
Improved				
Piped water	6 (3.4%)	11 (6.3%)	6 (3.4%)	8 (4.4%)
Borehole	151 (86.3%)	137 (78.3%)	146 (83.4%)	137 (75.7%)
Protected well	3 (1.7%)	6 (3.4%)	11 (6.3%)	7 (3.9%)
Non-improved				
Unprotected well	8 (4.6%)	11 (6.3%)	8 (4.6%)	21 (11.6%)
Lake	3 (1.7%)	5 (2.9%)	1 (0.6%)	6 (3.3%)
River, pond	0 (0.0%)	1 (0.6%)	1 (0.6%)	0 (0.0%)
Other/missing	4 (2.3%)	4 (2.3%)	2 (1.1%)	2 (1.1%)

Data are presented as mean SD, unless otherwise stated.
[†]Socio-economic score is derived from household assets and housing conditions.

Table 2 The prevalence of virus and parasite at each study visit by intervention group

		Number of positive samples by intervention group (%)				
	Visit	Control	CSB	Milk LNS	Soy LNS	p-Value[†]
Giardia lamblia	At enrolment (6 months)	10 (9)	10 (9)	9 (8)	14 (12)	0.764
	12 months	21 (29)	15 (19)	18 (22)	17 (22)	0.523
	18 months	25 (23)	29 (28)	32 (29)	30 (26)	0.729
Cryptosporidium (spp.)	At enrolment (6 months)	5 (5)	8 (7)	7 (7)	6 (5)	0.904
	12 months	6 (8)	8 (10)	6 (7)	4 (5)	0.724
	18 months	4 (4)	3 (3)	0 (0)	2 (2)	0.201
Enterovirus	At enrolment (6 months)	73 (67)	83 (72)	71 (66)	76 (67)	0.823
	12 months	58 (79)	61 (76)	64 (78)	68 (87)	0.309
	18 months	89 (81)	83 (80)	92 (84)	91 (79)	0.832
Parechovirus	At enrolment (6 months)	22 (21)	33 (28)	19 (18)	31 (27)	0.186
	12 months	12 (16)	13 (16)	12 (15)	13 (17)	0.986
	18 months	20 (18)	16 (15)	19 (17)	18 (16)	0.942
Rotavirus	At enrolment (6 months)	3 (3)	4 (3)	4 (4)	4 (4)	>0.999
	12 months	1 (1)	1 (1)	0 (0)	1 (1)	0.712
	18 months	0 (0)	1 (1)	2 (2)	0 (0)	0.279
Norovirus	At enrolment (6 months)	18 (17)	28 (24)	29 (27)	26 (23)	0.340
	12 months	16 (22)	15 (19)	20 (24)	16 (21)	0.847
	18 months	15 (14)	13 (13)	18 (16)	21 (18)	0.635
Rhinovirus	At enrolment (6 months)	33 (31)	29 (25)	29 (27)	34 (30)	0.738
	12 months	7 (10)	17 (21)	14 (17)	20 (26)	0.065
	18 months	31 (28)	37 (36)	41 (37)	29 (25)	0.158

CSB, Corn-soy blend.
Six months: Control N = 106, CSB N = 116, Milk LNS N = 107, Soy LNS N = 114; 12 months: Control N = 73, CSB N = 80, Milk LNS N = 82, Soy LNS N = 78; 18 months: Control N = 110, CSB N = 104, Milk LNS N = 110, Soy LNS N = 115.
[†]p-Value obtained with Fisher's exact test for individual visits.

education, season of visit, enrolment site, water source and socio-economic score. We performed statistical analyses using Stata 15.1 (Stata Corp, College Station, TX, USA).

RESULTS

Of the 1312 infants who were identified through community census, 472 were either ineligible, or they were not brought to an enrolment session (Fig. 1). At six months of age, the mean standard deviation (SD) LAZ of the participants was −1.75 (0.97) and the mean WLZ was 0.46 (1.05). Less than five per cent of the participants had access to a piped water, and 91% of the participants had a sanitary facility, a pit latrine. There were no differences in the background characteristics between the intervention groups (Table 1).

When all the time points and intervention groups were combined, the prevalence of tested microbes ranged from 2.0% of rotavirus to 75.9% of enterovirus. The percentage of positive stools for *G. lamblia* both at six and 12 months of age was 6.2%, the positivity both at 12 and 18 months of age was 10.6% and the positivity at six, 12 and 18 months of age was 5.1%. There was no statistically significant association between the dietary followed by the children and the prevalence of any of the studied microbes at any age (Table 2).

Because there were no differences in the prevalence of pathogens between the intervention groups, the subgroups were combined for the growth analyses. All age groups combined, children negative for *G. lamblia* had a mean SD of −0.01 (0.49) change in LAZ, whereas the figure was −0.12 (0.45) among those positive for *G. lamblia* (difference −0.10, 95% CI −0.21 to −0.00, p = 0.047). The LAZ change difference was largest and statistically significant (p = 0.042) between 18 and 21 months of age and smaller and statistically not significant at earlier time points. Children negative for rhinovirus at 12 months of age had a mean SD of −0.10 (0.51) change in LAZ, whereas the figure was −0.38 (0.50) among those positive for rhinovirus (difference −0.27, 95% CI −0.49 to −0.06, p = 0.011). With the exception of rhinovirus at this time point, the prevalence of other studied viruses was not associated with the children's linear growth in the subsequent three-month period (Table 3).

Generally, there was no association between the microbial prevalence at six, 12 or 18 months and attained LAZ by the same time point. The only exception was *Cryptosporidium* detection at 18 months of age when the mean (95% CI) LAZ was 0.87 (−1.64 to −0.10, p = 0.026) units lower among children with *Cryptosporidium* in their stool than those without it (Table 4).

DISCUSSION

The main aim of this study was to analyse if intestinal presence of *G. lamblia*, *Cryptosporidium* species, or

Table 3 The association between virus or parasite and change in LAZ in the subsequent three-month interval

		Covariate adjusted mean SD[†] change in the participants' LAZ during a three-month follow-up			
Virus/parasite	Child age (Change between)	Children without the indicated microbe	Children with the indicated microbe	Difference between groups	p-Value[‡]
Giardia lamblia	All ages combined	−0.01 (0.49)	−0.12 (0.45)	−0.10 (−0.21, −0.00)	0.047
	6–9 months	0.01 (0.48)	0.06 (0.46)	0.05 (−0.13, 0.24)	0.581
	12–15 months	−0.14 (0.51)	−0.25 (0.46)	−0.11 (−0.35, 0.13)	0.364
	18–21 months	0.01 (0.50)	−0.13 (0.46)	−0.14 (−0.27, −0.01)	0.042
Cryptosporidium spp.	All ages combined	−0.03 (0.49)	−0.03 (0.43)	−0.00 (−0.16, 0.16)	0.998
	6–9 months	0.02 (0.48)	−0.11 (0.45)	−0.13 (−0.36, 0.10)	0.253
	12–15 months	−0.17 (0.50)	−0.00 (0.44)	0.17 (−0.12, 0.47)	0.247
	18–21 months	−0.03 (0.50)	−0.37 (0.43)	−0.34 (−0.72, 0.04)	0.081
Enterovirus	All ages combined	−0.03 (0.48)	−0.04 (0.49)	−0.01 (−0.10, 0.08)	0.824
	6–9 months	−0.01 (0.49)	0.03 (0.48)	0.04 (−0.08, 0.17)	0.493
	12–15 months	−0.07 (0.50)	−0.17 (0.50)	−0.10 (−0.33, 0.14)	0.394
	18–21 months	−0.02 (0.49)	−0.04 (0.49)	−0.02 (−0.19, 0.15)	0.923
Parechovirus	All ages combined	−0.04 (0.50)	−0.01 (0.44)	0.03 (−0.06, 0.13)	0.512
	6–9 months	0.02 (0.49)	−0.01 (0.46)	−0.03 (−0.16, 0.11)	0.665
	12–15 months	−0.18 (0.52)	−0.06 (0.45)	0.12 (−0.12, 0.35)	0.336
	18–21 months	−0.05 (0.51)	−0.01 (0.45)	0.03 (−0.12, 0.18)	0.656
Rotavirus	All ages combined	−0.04 (0.49)	0.10 (0.41)	0.14 (−0.11, 0.39)	0.265
	6–9 months	0.01 (0.48)	0.14 (0.39)	0.13 (−0.18, 0.44)	0.423
	12–15 months	NA	NA	NA	NA
	18–21 months	NA	NA	NA	NA
Norovirus	All ages combined	−0.04 (0.49)	−0.02 (0.47)	0.02 (−0.09, 0.12)	0.762
	6–9 months	0.02 (0.49)	0.00 (0.46)	−0.01 (−0.15, 0.12)	0.825
	12–15 months	−0.16 (0.51)	−0.13 (0.51)	0.03 (−0.17, 0.24)	0.736
	18–21 months	−0.04 (0.50)	−0.02 (0.49)	0.02 (−0.14, 0.19)	0.765
Rhinovirus	All ages combined	−0.02 (0.48)	−0.08 (0.48)	−0.07 (−0.16, 0.02)	0.136
	6–9 months	0.01 (0.48)	0.02 (0.48)	0.01 (−0.11, 0.14)	0.830
	12–15 months	−0.10 (0.51)	−0.38 (0.50)	−0.27 (−0.49, −0.06)	0.011
	18–21 months	−0.03 (0.49)	−0.05 (0.49)	−0.02 (−0.15, 0.12)	0.818

Adjusted for child's virus or parasite status at enrolment, maternal height, maternal BMI at enrolment, child sex, maternal education, season of visit (Jan–Mar, Apr–Jun, Jul–Sep, Oct–Dec), enrolment site, water source and socio-economic score, principal component. All ages combined: N = 686 (N of participants 400). Six to nine months: N = 352. 12–15 months: N = 131. 18–21 months: N = 203.

[†]SD for adjusted means estimated from least squares regression.

[‡]p-Value and 95% CI obtained from least squares regression. For all time points combined, intragroup correlation due to multiple measurements per participant taken into account by using robust standard errors for clustered data.

enterovirus, rotavirus, norovirus, parechovirus or rhinovirus in early childhood is associated with subsequent growth restriction among children living in Sub-Saharan resource-poor areas. A secondary aim was to assess if dietary supplementation with LNS or corn-soy flour would be associated with microbial detection. In a sample of 840 asymptomatic children aged six to 18 months, there was a high prevalence of viruses and parasites. Primarily, the detection of *G. lamblia* was associated with a growth restriction in certain three-month periods. Dietary fortification with LNS or corn–soy flour was not associated with the prevalence of the studied viruses or parasites among the children.

The main possible causes of bias in our study design were the symptomatic infections and the contamination of the samples during the laboratory analyses. For this reason, the guardians recorded on a daily basis the presence or absence of various illness symptoms. To detect possible contamination during the laboratory analyses, we used negative control samples in sample preparation, extraction and PCR but we found none. Also, the possibility of false negative results was reduced by the use of the positive control samples.

The strength of the trial was the high internal validity. This was due to the random group allocation, blinding of the outcome assessors and similarity of the intervention groups at enrolment. The success of faecal sample collection was relatively low (47.4%) which can be considered as a limiting factor in the study. However, this should not have biased our conclusions as the proportions with available sample were similar in all the intervention groups, also at 12 months of age when we obtained the lowest sample collection rate. Accordingly, we conclude that the sample findings are valid and representative of the target population from which the sample was drawn.

Of the studied microbes in our study, the presence of *G. lamblia* was mainly negatively associated with subsequent linear growth among asymptomatic infants and

Table 4 The association between virus or parasite and attained LAZ

		Covariate adjusted mean SD† attained LAZ by the participants' PCR test result			
Virus/parasite	Child age	Children without the indicated microbe	Children with the indicated microbe	Difference between groups	p-Value‡
Giardia lamblia	All ages combined	−1.77 (0.93)	−1.86 (0.92)	−0.09 (−0.26, 0.08)	0.292
	6 months	−1.68 (0.95)	−1.51 (0.94)	0.17 (−0.13, 0.46)	0.276
	12 months	−1.71 (0.94)	−1.96 (0.93)	−0.25 (−0.61, 0.12)	0.182
	18 months	−2.00 (0.94)	−1.91 (0.96)	0.17 (−0.09, 0.44)	0.201
Cryptosporidium spp.	All ages combined	−1.79 (0.92)	−1.67 (0.99)	0.13 (−0.20, 0.45)	0.450
	6 months	−1.65 (0.95)	−1.87 (1.03)	−0.22 (−0.60, 0.15)	0.244
	12 months	−1.80 (0.94)	−1.35 (1.01)	0.45 (−0.11, 1.01)	0.113
	18 months	−2.00 (0.94)	−2.87 (0.99)	−0.87 (−1.64, −0.10)	0.026
Enterovirus	All ages combined	−1.76 (0.93)	−1.79 (0.93)	−0.03 (−0.18, 0.12)	0.683
	6 months	−1.66 (0.93)	−1.66 (0.96)	−0.00 (−0.19, 0.19)	0.974
	12 months	−1.88 (0.95)	−1.73 (0.94)	0.15 (−0.21, 0.51)	0.421
	18 months	−1.99 (0.94)	−2.02 (0.95)	−0.03 (−0.35, 0.29)	0.859
Parechovirus	All ages combined	−1.77 (0.93)	−1.83 (0.90)	−0.06 (−0.21, 0.09)	0.456
	6 months	−1.66 (0.96)	−1.68 (0.90)	−0.31 (−0.24, 0.18)	0.803
	12 months	−1.74 (0.95)	−1.89 (0.91)	−0.15 (−0.56, 0.25)	0.456
	18 months	−2.04 (0.95)	−1.95 (0.92)	0.09 (−0.21, 0.39)	0.554
Rotavirus	All ages combined	−1.79 (0.93)	−1.50 (0.80)	0.30 (−0.26, 0.86)	0.299
	6 months	−1.66 (0.95)	−1.62 (0.75)	0.05 (−0.43, 0.52)	0.847
	12 months	−1.76 (0.94)	−1.66 (0.87)	0.10 (−1.86, 2.06)	0.919
	18 months	−2.01 (0.95)	−3.34 (0.81)	−1.32 (−3.32, 0.68)	0.193
Norovirus	All ages combined	−1.78 (0.94)	−1.81 (0.86)	−0.03 (−0.19, 0.13)	0.670
	6 months	−1.63 (0.96)	−1.77 (0.93)	−0.14 (−0.35, 0.07)	0.193
	12 months	−1.77 (0.96)	−1.73 (0.86)	0.04 (−0.31, 0.38)	0.828
	18 months	−2.00 (0.95)	−2.10 (0.91)	−0.10 (−0.42, 0.22)	0.532
Rhinovirus	All ages combined	−1.77 (0.92)	−1.83 (0.95)	−0.07 (−0.24, 0.11)	0.462
	6 months	−1.65 (0.95)	−1.71 (0.96)	−0.06 (−0.25, 0.13)	0.540
	12 months	−1.76 (0.93)	−1.80 (0.96)	−0.05 (−0.43, 0.33)	0.797
	18 months	−2.00 (0.93)	−2.06 (0.97)	−0.05 (−0.31, 0.21)	0.696

Adjusted for child's virus or parasite status at enrolment, maternal height, maternal BMI at enrolment, child sex, maternal education, season of visit (Jan–Mar, Apr–Jun, Jul–Sep, Oct–Dec), enrolment site, water source and socio-economic score. All ages combined: N = 861 (N of participants 451). Six months: N = 426. 12 months: N = 190. 18 months: N = 245.

†SD for adjusted means estimated from least squares regression.

‡p-Value and 95% CI obtained from least squares regression. For all time points combined, intragroup correlation due to multiple measurements per participant taken into account by using robust standard errors for clustered data.

young children. Such an association between *G. lamblia* and child growth has earlier been identified in cohort studies in Sub-Saharan Africa, Southern Asia and South America (17). Although there have also been studies in which this association was not found (18,19), the bulk of the evidence seems to suggest a link between symptomatic or asymptomatic *Giardia* infection and reduced linear growth in most low-income contexts. Such an association is likely to be causal, as *Giardia* infections are often chronic (20) and can lead to prolonged aberrations in gut function and nutrient absorption (21,22). Additionally, systemic inflammation caused by chronic infections can interfere the growth hormone – insulin-like growth factor 1 pathway and reduce growth plate activity and hence linear growth (21,23).

In our sample, asymptomatic *Cryptosporidium* spp. carriage was associated with reduced linear growth only at 18 months of age. A recent meta-analysis of data from six studies suggested a linear growth restriction associated both with symptomatic or asymptomatic *Cryptosporidium* infection (24).

Short episode duration may be the reason why in most cases, there was no association between the documented viral infections and children's length gain in the subsequent three-month period. The excretion of enterovirus, parechovirus, rotavirus, norovirus and rhinovirus typically lasts only some days to weeks. Furthermore, viral infections often elicit an interferon-based host response that provides short-acting protection also towards other viral species (25). A single time point assessment of a child's viral infection status is thus unlikely to be representative of her situation over the whole subsequent period of three months. If this was the case, the use of three-month follow-up could obscure the importance of viral infections in linear growth restriction. Few studies have addressed the association between asymptomatic viral infections and growth (26,27). To better assess the growth impact of non-chronic viral infections, one should thus probably use with a short follow-up period a more sensitive outcome variable, such as knemometry, plasma

concentrations of insulin-like growth factor or collagen X (21,28,29).

The finding of no association between the dietary supplement given to the children and detection of selected microbes in the children's stool is not surprising, since diet has not been reported to significantly influence viral propagation in children who are not malnourished. The result is also in line with an earlier intervention study, in which provision of LNS to children with severe acute malnutrition was not associated with a change in the detected virome in the children's stools (30). In a previously published research on the same study sample, the dietary intervention was not either associated with the composition of the children's microbiota (10).

Taken together, our results suggest that asymptomatic *G. lamblia* infections may restrict child growth in low-income settings such as rural Malawi. As a conclusion, asymptomatic viral infections are common and may also influence growth, but more refined measurement of growth or growth-related activity is necessary to better elucidate this question.

ACKNOWLEDGEMENTS

We thank the study participants and the staff members of the cooperative research groups and Lungwena Health Center for their help and support in all stages of the study. We thank Maria Ovaskainen, Eveliina Jalonen, Marie Hélou and Emily McKinney for their technical assistance and Eeli Jaakkola and Juha Pyykkö for their help with the research data.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

FUNDING

The LCNI-5 clinical trial was funded by the Academy of Finland, grants 109796 and 127025, with additional funding from the Foundation for Paediatric Research in Finland, the competitive research funding of the Tampere University Hospital, grants 9H003 and 9J006, and the U.S. Agency for International Development under terms of cooperative agreement GHN-A-00-08-00001-00, through the Food and Nutrition Technical Assistance-2 Project managed by the Academy for Educational Development and the Nutriset Inc.

References

1. UNICEF/WHO/World Bank Group. Levels and trends in child malnutrition. Joint Child Malnutrition Estimates. Key findings of the 2017 edition.
2. Humphrey JH. Child undernutrition, tropical enteropathy, toilets, and handwashing. *Lancet* 2009; 374: 1032–5.
3. Trehan I, Kelly P, Shaikh N, Manary M. New insights into environmental enteric dysfunction. *Arch Dis Child* 2016; 101: 741–4.
4. Caulfield LE, MAL-ED Network Investigators. Relationship between growth and illness, enteropathogens and dietary intakes in the first 2 years of life: findings from the MAL-ED birth cohort study. *BMJ Glob Health* 2017; 2: e000370.
5. Lim Y, Ahmad R, Smith H. Current status and future trends in Cryptosporidium and Giardia epidemiology in Malaysia. *J Water Health* 2008; 6: 239–54.
6. Siwila J, Phiri IGK, Enemark HL, Nchito M, Olsen A. Intestinal helminths and protozoa in children in pre-schools in Kafue district, Zambia. *Trans R Soc Trop Med Hyg* 2010; 104: 122–8.
7. Acosta A, Chavez C, Flores J, Olotegui M, Pinedo S, Trigoso DR, et al. The MAL-ED study: a multinational and multidisciplinary approach to understand the relationship between enteric pathogens, malnutrition, gut physiology, physical growth, cognitive development, and immune responses in infants and children up to 2 years of age in resource-poor environments. *Clin Infect Dis* 2014; 59(Suppl. 4): S193–206.
8. Jones A, Rukobo S, Chasekwa B, Mutasa K, Ntozini R, Mbuya M, et al. Acute illness is associated with suppression of the growth hormone axis in Zimbabwean infants. *Am J Trop Med Hyg* 2015; 92: 463–70.
9. Mangani C, Maleta K, Phuka J, Cheung YB, Thakwalakwa C, Dewey K, et al. Effect of complementary feeding with lipid-based nutrient supplements and corn-soy blend on the incidence of stunting and linear growth among 6- to 18-month-old infants and children in rural Malawi. *Matern Child Nutr* 2015; 11(Suppl 4): 132–43.
10. Cheung YB, Xu Y, Mangani C, Fan YM, Dewey KG, Salminen SJ, et al. Gut microbiota in Malawian infants in a nutritional supplementation trial. *Trop Med Int Health* 2016; 21: 283–90.
11. Aakko J, Endo A, Mangani C, Maleta K, Ashorn P, Isolauri E, et al. Distinctive intestinal lactobacillus communities in 6-month-old infants from Rural Malawi and Southwestern Finland. *J Pediatr Gastroenterol Nutr* 2015; 61: 641–8.
12. Fan Y-M, Oikarinen S, Lehto K-M, Nurminen N, Juuti R, Mangani C, et al. High prevalence of selected viruses and parasites and their predictors in Malawian children. *Epidemiol Infect* 2019; 147: e90.
13. Krogvold L, Edwin B, Buanes T, Frisk G, Skog O, Anagandula M, et al. Detection of a low-grade enteroviral infection in the islets of langerhans of living patients newly diagnosed with type 1 diabetes. *Diabetes* 2015; 64: 1682–7.
14. Honkanen H, Oikarinen S, Peltonen P, Simell O, Ilonen J, Veijola R, et al. Human rhinoviruses including group C are common in stool samples of young Finnish children. *J Clin Virol* 2013; 56: 250–4.
15. Nurminen N, Juuti R, Oikarinen S, Fan YM, Lehto KM, Mangani C, et al. High-throughput multiplex quantitative polymerase chain reaction method for *Giardia lamblia* and Cryptosporidium species detection in stool samples. *Am J Trop Med Hyg* 2015; 92: 1222–6.
16. Child WHO. Growth standards based on length/height, weight and age. *Acta Paediatr Suppl* 2006; 450: 76–85.
17. Rogawski ET, Bartelt LA, Platts-Mills JA, Seidman JC, Samie A, Havt A, et al. Determinants and impact of Giardia infection in the first 2 years of life in the MAL-ED birth cohort. *J Pediatric Infect Dis Soc* 2017; 6: 153–60.
18. Veenemans J, Mank T, Ottenhof M, Baidjoe AY, Mbugi EV, Demir AY, et al. Protection against diarrhea associated with *Giardia intestinalis* is lost with multi-nutrient supplementation: a study in Tanzanian children. *PLoS Negl Trop Dis* 2011; 5: e1158.
19. Mondal D, Minak J, Alam M, Liu Y, Dai J, Korpe P, et al. Contribution of enteric infection, altered intestinal barrier function, and maternal malnutrition to infant malnutrition in Bangladesh. *Clin Infect Dis* 2012; 54: 185–92.

20. Svärd SG, Ankarklev J, Troell K, Jerlström-Hultqvist J, Ringqvist E. Behind the smile: cell biology and disease mechanisms of Giardia species. *Nat Rev Microbiol* 2010; 8: 413–22.
21. Walters TD, Griffiths AM. Mechanisms of growth impairment in pediatric Crohn's disease. *Nat Rev Gastroenterol Hepatol* 2009; 6: 513–23.
22. Minetti C, Chalmers RM, Beeching NJ, Probert C, Lamden K. Giardiasis. *BMJ* 2016; 355: i5369.
23. Ashorn P, Vanhala H, Pakarinen O, Ashorn U, De Costa A. Prevention of intrauterine growth restriction and preterm birth with presumptive antibiotic treatment of pregnant women: a literature review. *Nestle Nutr Inst Workshop Ser* 2015; 81: 37.
24. Khalil IA, Troeger C, Rao PC, Blacker BF, Brown A, Brewer TG, et al. Morbidity, mortality, and long-term consequences associated with diarrhoea from Cryptosporidium infection in children younger than 5 years: a meta-analyses study. *Lancet Glob Health* 2018; 6: e758–68.
25. Fensterl V, Sen GC. Interferons and viral infections. *BioFactors* 2009; 35: 14–20.
26. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* 2013; 382: 209–22.
27. Rogawski ET, MAL-ED Network Investigators. Use of quantitative molecular diagnostic methods to investigate the effect of enteropathogen infections on linear growth in children in low-resource settings: longitudinal analysis of results from the MAL-ED cohort study. *Lancet Glob Health* 2018; 6: 1319–28.
28. Urlacher SS, Snodgrass JJ, Liebert MA, Cepon-Robins T, Gildner TE, Sugiyama LS. The application of knemometry to measure childhood short-term growth among the indigenous Shuar of Ecuador. *Am J Phys Anthropol* 2016; 160: 353–7.
29. Coghlan R, Oberdorf J, Sienko S, Aiona M, Boston B, Connelly K, et al. A degradation fragment of type X collagen is a real-time marker for bone growth velocity. *Sci Transl Med* 2017; 9: eaan4669.
30. Reyes A, Blanton LV, Cao S, Zhao G, Manary M, Trehan I, et al. Gut DNA viromes of Malawian twins discordant for severe acute malnutrition. *Proc Natl Acad Sci U S A* 2015; 112: 11941–6.

