Infant growth faltering linked to subclinical mastitis, maternal faecal–oral contamination, and breastfeeding

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Abstract

Infant growth faltering occurs in breastfed infants <6 months of age. The possibility that maternal health status contributes to this growth faltering is underexplored. We investigated whether (a) subclinical mastitis (SCM), an asymptomatic inflammation of the breast, (b) maternal intestinal nematode and protozoan infections, indicators of faecal–oral contamination, or (c) poor breastfeeding practices increased the odds of stunting (length-for-age z-score < −2SD), underweight (weight-for-age z-score < −2SD), or low head circumference (head circumference-for-age z-score < −2SD) in breastfed infants in rural indigenous communities in Guatemala. Mother–infant dyads ($n = 105$) were subdivided into those with and without SCM (milk Na:K ratio > 0.6). Maternal and infant anthropometry were measured at the time of breast milk collection. Maternal stool samples were examined for the presence of intestinal nematodes and protozoa. Questionnaires were used to characterize breastfeeding practices (exclusivity and frequency) and support, hygiene (latrine use and household faucet), and infant diarrhoea. SCM occurred in 14% of women and was associated with increased odds of infant stunting (odds ratio [OR] = 4.3; confidence interval [CI] [1.1, 15.8]), underweight (OR = 9.2; CI [1.8, 48.0]), and low head circumference (OR = 15.9; CI [2.6, 96.9]). Maternal pathogenic protozoa and nematodes were uncommon (<4%), but nonpathogenic protozoa were common (e.g., *Entamoeba coli* [39%]). *Entamoeba coli* increased the likelihood (OR = 3.3; CI [1.02, 10.6]) of low head circumference, whereas higher breastfeeding frequency lowered its odds (OR = 0.74, CI [0.56, 0.97]). Prevention of SCM may improve early infant growth, but public health measures that increase breastfeeding frequency and reduce faecal–oral contamination may be required to minimize low head circumference.

KEYWORDS

breastfeeding frequency, *Entamoeba coli*, Guatemala, infant growth, low head circumference, subclinical mastitis

1 | INTRODUCTION

Infant growth faltering is recognized as a worldwide problem (Victora, de Onis, Hallal, Blössner, & Shrimpton, 2010), but emerging evidence that growth faltering occurs in breastfed infants less than 6 months of age has heightened international concerns (Kerac et al., 2011;

Martorell & Young, 2012; Rehman et al., 2009). Previous studies using National Center for Health Statistics (NCHS) growth reference curves had identified 12–23 months as the most vulnerable time period (de Onis, Garza, Onyango, & Borghi, 2007), but the application of the World Health Organization (WHO) Growth Standards to large nationally representative samples from India and Guatemala has revealed that

growth faltering in breastfed infants less than 5 months had been underestimated with infant stunting now exceeding 20% in both countries and wasting having tripled in India (Martorell & Young, 2012).

Explanations for infant growth faltering prior to 6 months of age are limited as studies have rarely focused on this age group. Factors such as low-birthweight (Mosites, Dawson-Hahn, Walson, Rowhani-Rahbar, & Neuhauser, 2017) and household poverty (Martorell & Young, 2012) are accepted correlates of growth faltering before 2 years. There is also some evidence that exposure of infants as young as 3 months to intestinal protozoa is associated with growth faltering at 2 years (Goto, Mascie-Taylor, & Lunn, 2009) and more than five episodes of diarrhoea before 2 years contributes to stunting (Mosites et al., 2017). However, the relationship of environmental enteropathy to growth faltering and stunting in young infants is complex and not fully understood (Keusch et al., 2013; MAL-ED Network Investigators, 2014). There is evidence that a maternal intestinal parasitic infection can retard linear growth in neonatal mice (Odiere, Scott, Weiler, & Koski, 2010). Recently, it was proposed that the considerable fraction of unexplained variability in early infant growth faltering may relate to a more prominent role of maternal health and breastfeeding practices than previously recognized (Mosites et al., 2017; Rieger & Trommlerová, 2016).

One understudied maternal condition is subclinical mastitis (SCM). SCM, which is an asymptomatic inflammatory condition of the lactating breast usually diagnosed using an elevated milk Na:K ratio, has been associated with growth faltering before 6 months of age (Aryeetey, Marquis, Brakohiapa, Timms, & Lartey, 2009; Gomo et al., 2003; Makasa et al., 2007). Milk stasis contributes to SCM because it creates ideal conditions for bacterial overgrowth and breast inflammation (WHO, 2000). Stasis usually occurs when breast milk is not efficiently removed because of restricted flow due to poor infant feeding practices or because reduced breastfeeding frequency or duration lowers milk volume (WHO, 2000). SCM has been associated with poor maternal and breast health and nonexclusive breastfeeding (Kasonka et al., 2006) and with compositional changes in breast milk (Eriksen, Christensen, Lind, & Michaelsen, 2018; Li, Solomons, Scott, & Koski, 2016, 2018). Finally, poor sanitation and hygiene practices have been implicated (Contreras & Rodríguez, 2011); although interventions to improve maternal hand washing have reduced infant diarrhoea, these practices did not improve infant growth (Langford, Lunn, & Panter-Brick, 2011).

Lack of access to suitable water and sanitation infrastructure is a common experience for breastfeeding mothers in developing countries. This creates an unhygienic environment where mothers and their infants may be exposed to faecal-oral transmission of pathogens (Keusch et al., 2013; Mbuya & Humphrey, 2016) and intestinal protozoans normally considered as nonpathogenic including *Entamoeba coli* and *Blastocystis hominis*. These nonpathogenic protozoans are used as indicators of poor hygiene and sanitation given their association with poverty (Ignacio et al., 2017), poor water quality (Hemmati et al., 2017), and faecal contamination of soil and water that can lead to transmission both through faecal-oral routes (Fletcher, Stark, Harkness, & Ellis, 2012) and via mechanical vectors such as flies (Adenusi & Adewoga, 2013) and contaminated fresh food (Mohamed, Siddig, Elaagip, Edris, & Nasr, 2016). A recent meta-analysis showed

Key messages

- Subclinical mastitis may be an underappreciated determinant of underweight, stunting, and low head circumference in breastfed infants prior to 6 months of age.
- Promoting higher breastfeeding frequency may be beneficial in reducing the risk of low head circumference even in populations where breastfeeding frequency falls within norms.
- Public health professionals may be interested in our observation that maternal *Entamoeba coli* increased the likelihood of low head circumference suggesting that faecal-oral contamination may be associated with early brain development.
- This paper expands the paradigm for early infant growth faltering from a focus on infant diarrhoea and nutrition to one that incorporates maternal factors.

that availability and use of sanitation facilities and water treatment lowered the odds of parasitic protozoan infection and that water treatment also lowered odds of *B. hominis* in children and adults (Speich, Croll, Fürst, Utzinger, & Keiser, 2016). There is no evidence, however, that nonpathogenic protozoans in mothers or infants are associated with impaired growth of infants <6 months of age.

To date, research exploring the determinants of infant growth faltering has not simultaneously evaluated the contribution of breastfeeding practices, SCM, and indicators of sanitation and hygiene, even though regions where SCM and infant growth faltering coexist typically have poor sanitation. The study was conducted in the Western Highlands of Guatemala where 40–52% of infants under 6 months of age are stunted, 15.5% are underweight, and 22.8% have low head circumference (Chomat et al., 2015), where SCM has been reported (Li et al., 2018) and where breastfeeding practices have been described (Wren, Solomons, Chomat, Scott, & Koski, 2015). The objective of this study was to determine if SCM, poor breastfeeding practices, and maternal faecal-oral contamination as measured by intestinal nematodes and protozoa were associated with higher odds of stunting, underweight, and low head circumference.

2 | METHODS

2.1 | Study design, recruitment, and ethical considerations

A cross-sectional study of 105 breastfeeding mother–infant dyads was conducted in eight rural Mam-Mayan indigenous communities. Between June 2012 and January 2013, *comadronas* (traditional midwives) and community healthcare workers (CHWs) recruited breastfeeding mother–infant dyads at 0–6 weeks or 4–6 months of age. Of the 123 mothers initially enrolled, 117 (94%) provided breast

milk samples at either 0–6 weeks or 4–6 months based on infant age. Mothers with infants <4 days ($n = 4$) and mothers with breast milk volumes insufficient for triplicate analysis ($n = 8$) were excluded. Complete data were available for 105 breastfeeding mother–infant dyads with almost an equal number of infants at 0–6 weeks ($n = 52$, mean age = 21.1 ± 10.6 days; early lactation) and 4–6 months ($n = 53$, mean age = 147.7 ± 18.3 days; established lactation) post-partum. Institutional ethical approval was obtained, and all mothers provided fully informed consent.

2.2 | Questionnaires

Trained CHWs administered the questionnaire orally in either Spanish or *Mam* to participants during a 30-min interview. Socio-demographics (household assets), maternal characteristics (age and parity), maternal health (headache and stomach ache), infant health (diarrhoea in past week), and sanitation infrastructure (household faucet and pit latrine) were collected. Breastfeeding practices (exclusive, predominant, or mixed feeding; WHO, 2003), breastfeeding frequency, and breastfeeding support (provider and problems) were queried by CHWs.

2.3 | Anthropometry

Detailed methodology has been previously published (Chomat et al., 2015). In brief, two trained Guatemalan nutritionists measured maternal weight (kg), height (cm) and infant length, and weight and head circumference in triplicate using an infantometer (SECA 210), a digital infant scale (SECA 354), and a head circumference baby band (SECA 212), respectively. These anthropometric measures were completed on the same day as breast milk collection. Infant age was calculated using date of birth recorded on the mother's health card or in the absence of a card or as reported by the mother. Maternal body mass index (BMI) was calculated. Infants were classified as stunted (length-for-age z-score [LAZ] < $-2SD$), underweight (weight-for-age z-score [WAZ] < $-2SD$), wasted (weight-for-height z-score [WHZ] < $-2SD$), or having low head circumference (head circumference-for-age z-score [HCAZ] < $-2SD$) using WHO Anthro Software (3.1) according to WHO Growth Reference Standards (WHO, 2006).

2.4 | Maternal faecal collection and analysis

A single stool sample was analysed using direct smear by an experienced laboratory technician. Presence of intestinal nematodes (*Ascaris*, *Trichuris*, hookworm) and both pathogenic (*Giardia* spp, *Entamoeba histolytica*, and *Entamoeba dispar*) and nonpathogenic (*B. hominis*, *Entamoeba coli*, *Endolimax nana*, and *Iodamoeba butschlii*) protozoa were recorded.

2.5 | Subclinical mastitis

A single, unilateral breast milk sample, from the breast not recently used for feeding, was collected under the supervision of a trained *comadrona*. Milk samples were analysed in triplicate for Na and K

using a Varian ICP-820MS (Analytik Jena, Germany) equipped with a Collision Reaction Interface including calibration standards, internal and external controls, and reagent blanks. The lower limits of detection were $1.816 \mu\text{g L}^{-1}$ for Na and $4.887 \mu\text{g L}^{-1}$ for K. Mothers with breast milk Na:K ratio > 0.6 were classified as having SCM based on this cut-off indicating subclinical breast inflammation. The ratio is advantageous because it permits the use of milk samples without consideration of time of sampling or time since the infant was last fed; it controls for distribution of Na and K between aqueous and lipid phases of milk and declining concentrations of both Na and K throughout lactation (Filteau et al., 1999; Kasonka et al., 2006). Researchers had also shown less than a 5% discordance in SCM between breasts (Kasonka et al., 2006).

2.6 | Statistical analysis

Data were analysed using IBM SPSS statistical software version 25.0 (SPSS Inc./IBM Chicago IL, USA). Descriptive statistics (mean $\pm$ SD, percentages $\pm$ 95% CI) were computed. Chi-squares (χ^2) or Fisher's exact test (categorical variables) and Student's t-tests (continuous variables) were used to compare variables between mothers with and without SCM and between infants with normal anthropometric scores and those that were stunted, underweight, or had low head circumference. Multiple logistic regression analysis was used to examine hypothesized associations of SCM, breastfeeding practices, indicators of faecal–oral contamination with infant stunting, underweight, and low head circumference while controlling for maternal anthropometry and lactation stage. To build individual anthropometric models, variables were grouped into common themes: maternal delivery (frequency of antenatal care, delivery location), maternal characteristics (age, age at first pregnancy, weight, height, BMI, marital status, highest level of education, parity, occupation, and language), maternal intestinal protozoa (*B. hominis*, *Entamoeba coli*, *Endolimax nana*, and *I. butschlii*), infant health (diarrhoea and fever), infant sex (male and female), sanitation infrastructure (own faucet for water and pit latrine), maternal health (fever, headache, stomach ache, cough, flu, diarrhoea, and pyuria), socio-demographics (household assets), and breastfeeding practices (frequency per day and night, exclusive breastfeeding, and breastfeeding support). Highly correlated variables ($P > 0.7$, Variable Inflation Factor (VIF) > 10) within each theme were not tested in the same model to avoid multicollinearity. Only those with $P \leq 0.25$ were entered, and noncorrelated variables from each theme were entered into each anthropometric model. The attributable risk of SCM for each anthropometric measurement was assessed. For all logistic regression models, multicollinearity was tested and the Nagelkerke R^2 and the Hosmer and Lemeshow test for goodness of fit were reported (Lemeshow & Hosmer, 1982). Variables in the final model with $P < 0.05$ were considered significant.

3 | RESULTS

The prevalence of infant stunting, underweight, low head circumference, and wasting was 46%, 14%, 19%, and 2%, respectively. Fourteen percent of mothers had SCM (Na:K ratio > 0.6), and as expected, the

breast milk Na:K ratio was higher for mothers with SCM compared with mothers without SCM 147 (0.75 ± 0.15 vs. 0.41 ± 0.08 , $P < 0.001$). Mothers had a very low prevalence of *Ascaris lumbricoides* (2%), *Giardia* spp. (3%), and *E. histolytica/Entamoeba dispar*. (1%), and these infections were not considered further. In comparison, 70% of mothers had one or more nonpathogenic intestinal protozoa: *I. butschli* (12%), *B. hominis* (19%), *E. nana* (25%), and *Entamoeba coli* (39%). Almost all mothers (83%) had their own faucet for a water source, and most (84%) used a pit latrine compared with a toilet (16%).

Mothers with SCM were older ($P = 0.006$) and had higher parity ($P = 0.005$) compared with mothers without SCM (Table 1). Total breastfeeding frequency/24 hr ($P = 0.021$), specifically frequency during the daytime ($P = 0.008$), was slightly higher for mothers with SCM compared with mothers without SCM. Maternal health status,

intestinal protozoa, and indicators of poor sanitation did not differ between mothers with and without SCM, although a nonsignificant trend was observed for mothers with SCM who were less likely to have a home faucet ($P = 0.072$) and more likely to have stool *B. hominis* ($P = 0.095$). A higher percentage of mothers with SCM had underweight infants ($P = 0.023$) and infants with low head circumference ($P = 0.027$). Mothers with SCM had infants with lower WAZs ($P = 0.043$) and a nonsignificant trend towards lower height-for-age z-score ($P = 0.083$; Table 1).

Mothers with stunted infants had lower maternal weight ($P = 0.015$) and were shorter ($P = 0.012$) compared with mothers with nonstunted infants (Table 2). Mothers with infants having a low head circumference had lower maternal weight ($P = 0.008$) and lower BMI ($P = 0.008$) compared with mothers whose infants did not have a

TABLE 1 A comparison of factors based on subclinical mastitis status of mothers^{a,b}

	Na:K ratio ≤ 0.6 Non-SCM (n = 90)	Na:K ratio > 0.6 SCM (n = 15)	P value
Maternal characteristics			
Age, years	23.9 $\pm$ 6.2	28.8 $\pm$ 6.4	0.006
Weight, kg	51.4 $\pm$ 7.9	52.4 $\pm$ 8.1	0.656
Height, cm	146.6 $\pm$ 5.1	148.2 $\pm$ 3.9	0.264
Body mass index, kg m ⁻²	23.9 $\pm$ 3.2	23.8 $\pm$ 3.3	0.949
Parity, #	2.5 $\pm$ 2.0	4.2 $\pm$ 3.0	0.005
Maternal intestinal protozoa, % ^c			
<i>Blastocystis hominis</i>	17 [9.4, 26.3]	36 [12.7, 64.8]	0.095
<i>Entamoeba coli</i>	39 [28.8, 50.5]	36 [12.7, 64.8]	0.800
<i>Endolimax nana</i>	27 [18.2, 38.2]	7 [0.1, 33.8]	0.103
<i>Iodamoeba butschli</i>	14 [9.5, 24.4]	0 [0, 20.4]	0.131
Infant health			
Diarrhoea (% yes)	22 [14.3, 32.5]	53 [26.5, 78.7]	0.434
Sanitation infrastructure			
Own faucet for water (% yes)	86 [76.5, 92.0]	67 [38.3, 88.1]	0.072
Pit latrine (% yes)	86 [76.5, 92.0]	73 [44.9, 92.2]	0.234
Breastfeeding practices			
Frequency/24 hr, (#) ^d	12.0 $\pm$ 4.2	15.0 $\pm$ 6.0	0.021
Frequency/day, (#) ^d	7.1 $\pm$ 9.4	9.4 $\pm$ 3.7	0.008
Frequency/night, (#) ^d	4.8 $\pm$ 2.3	5.6 $\pm$ 3.3	0.284
Exclusive breastfeeding (% yes)	47 [36.0, 57.4]	60 [32.2, 83.6]	0.339
Infant characteristics			
Age, days	88.3 $\pm$ 64.1	64.7 $\pm$ 71.4	0.197
HAZ	-1.82 $\pm$ 1.21	-2.47 $\pm$ 1.92	0.083
Stunted (HAZ $< -2SD$), %	43 [32.9, 54.2]	60 [32.2, 83.6]	0.230
WAZ	-0.87 $\pm$ 0.90	-1.43 $\pm$ 1.38	0.043
Underweight (WAZ $< -2SD$), %	11 [5.4, 19.4]	33 [11.8, 61.6]	0.023
HCAZ ^e	-0.41 $\pm$ 4.27	-0.89 $\pm$ 1.49	0.670
Low head circumference (HCAZ $< -2SD$), % ^e	16 [8.7, 24.7]	40 [16.3, 67.7]	0.027

Note. SCM: subclinical mastitis; CI: confidence interval; HAZ: height-for-age z-score; HCAZ: head circumference-for-age z-score; SD, standard deviation; WAZ, weight-for-age z-score.

^aResults are presented as Mean $\pm$ SD or % (95% CI).

^bThe following additional factors did not differ ($P \geq 0.05$) between non-SCM and SCM mothers (data not shown): maternal education, marital status, occupation, language, headache, stomachache, pyuria, infant sex, and fever.

^cStool nonpathogenic protozoa (n = 98).

^dFeeding frequency (n = 100).

^eHCAZ and low head circumference (n = 104).

TABLE 2 A comparison of factors based on infant stunting, underweight, and low head circumference status^{a,b}

	Stunted		Underweight		Low head circumference	
	No (n = 57)	Yes (n = 48)	No (n = 90)	Yes (n = 15)	No (n = 84)	Yes (n = 20)
Maternal characteristics						
Age, years	24.6 ± 6.4	24.7 ± 6.6	24.4 ± 6.3	26.2 ± 7.2	24.7 ± 6.5	24.5 ± 6.7
Weight, kg	53.3 ± 7.2 ^A	49.5 ± 8.4 ^B	51.6 ± 7.8	51.1 ± 8.8	52.6 ± 7.9 ^A	47.4 ± 7.1 ^B
Height, cm	148.0 ± 4.3 ^A	145.6 ± 5.3 ^B	146.9 ± 5.0	147.2 ± 5.3	147.1 ± 4.9	146.0 ± 5.3
BMI, kg m ⁻¹	24.3 ± 3.0	23.3 ± 3.5	23.9 ± 3.3	23.5 ± 2.9	24.3 ± 3.3 ^A	22.2 ± 2.5 ^B
Parity, #	3.0 ± 2.5	2.5 ± 2.0	2.8 ± 2.3	2.7 ± 1.8	2.8 ± 2.3	2.9 ± 2.1
SCM (% yes)	11 [5, 21]	19 [10, 32]	11 [6, 19] ^B	33 [15, 59] ^A	11 [6, 19] ^B	30 [14, 52] ^A
Maternal intestinal Protozoa, %						
<i>Blastocystis hominis</i>	25 [15, 38]	13 [6, 26]	22 [14, 32]	7 [1, 30]	21 (13, 31)	15 (5, 36)
<i>Entamoeba coli</i>	42 [29, 55]	36 [23, 50]	40 [30, 51]	33 [15, 58]	34 (24, 45) ^B	60 (39, 78) ^A
<i>Endolimax nana</i>	32 [21, 45]	16 [8, 29]	25 [17, 36]	20 [7, 45]	26 [17, 37]	15 [5, 36]
<i>Iodamoeba butschli</i>	15 [8, 28]	9 [3, 20]	16 [9, 25]	0 [0, 24]	14 [8, 23]	5 [0, 25]
Breastfeeding practices						
Frequency, #/24 hr	12.6 ± 4.9	12.4 ± 4.3	12.6 ± 4.8	11.7 ± 3.6	12.9 ± 4.8 ^A	10.8 ± 3.6 ^B

^aResults are presented as Mean ± SD or % (95% CI).

^bDifferent uppercase superscripts denote statistical significance ($P < 0.05$) with exact P values in text.

low head circumference. Interestingly, *Entamoeba coli* was more common in mothers whose infants had low head circumference ($P = 0.033$), and mothers breastfed less frequently ($P = 0.041$) than mothers of infants with normal HCAZ.

Multiple logistic regression models were created for infant stunting, underweight, and low head circumference (Table 3). With regard to stunting, SCM was associated with higher odds of infant stunting (odds ratio [OR] = 4.28; $P = 0.029$) whereas higher maternal height was associated with lower odds of stunting (OR = 0.88; $P = 0.007$). Overall, 17% (0.17; [0.16, 0.18]) of stunting was attributed to SCM. With regard to underweight, SCM (OR = 9.21; $P = 0.008$) was the only variable associated with higher odds of infant underweight. Overall, 22% (0.22; [0.21, 0.23]) of underweight was attributed to SCM. With regard to low head circumference, SCM (OR = 15.89; $P = 0.003$) and maternal stool *Entamoeba coli* (OR = 3.28, $P = 0.047$) were associated with higher odds of infant low head circumference whereas higher breastfeeding frequency/day was associated with lower odds of low head circumference (OR = 0.74, $P = 0.031$). Overall, 54% (0.54; [0.52, 0.54]) of low head circumference was attributed to SCM.

4 | DISCUSSION

Despite earlier emphasis on dietary interventions, prevention of infections and diarrhoea, and improved sanitation and breastfeeding practices, infant growth faltering remains a challenge in low- and middle-income countries (Mosites et al., 2017) in part because of a lack of understanding of the contributory factors in breastfed infants less than 6 months of age. Our results showed that SCM increased the odds of stunting, underweight, and low head circumference. They also provide preliminary evidence that maternal health status during early lactation may be an underappreciated factor associated with infant growth faltering in breastfed infants prior to 6 months of age. Our results also revealed that the presence of maternal nonpathogenic

protozoans were associated with an increased likelihood of low head circumference and showed that higher breastfeeding frequencies were associated with a lower risk of low head circumference. Together, these findings provide novel insights into how maternal health and breastfeeding practices may contribute to early infant growth faltering in the first 6 months of life.

Our finding that SCM was an independent determinant of infant underweight and stunting is consistent with the negative association of Na:K ratio in multiple regression analysis with infant WAZ and LAZ at 6 weeks of age and with WAZ at 16 weeks in Zambia (Makasa et al., 2007) and with the lower WAZ in infants of Ghanaian mothers with Na:K > 1.0 despite similar milk volumes (Aryeetey et al., 2009). To our knowledge, ours is the first study to report that SCM also increased the odds of low head circumference. Three mechanisms have been proposed to link SCM with infant growth faltering in breastfed infants: lowered milk intake due to stasis (WHO, 2000), altered nutritive value of milk (Fetherston, Lai, & Hartmann, 2006; Li et al., 2016; Li et al., 2018), and presence of proinflammatory cytokines in milk (Millward, 2017). Among these, we may consider ruling out lowered milk intake due to stasis given that mothers did not report problems with breastfeeding and both mothers with and without SCM continued breastfeeding without reporting insufficient milk volume or obstruction of milk flow. Moreover, the possibility that SCM altered the nutritive value of milk is supported by findings that show lower milk lactose (Fetherston et al., 2006), lower milk phosphorus, and higher milk selenium (Li et al., 2018) and higher concentrations of milk serum IgA and lactoferrin (Filteau et al., 1999). Furthermore, it has been reported that the Na:K ratio was associated with higher concentrations of milk cytokines (Filteau et al., 1999; Li et al., 2018) and differences in milk mineral and trace element concentrations (Li et al., 2018). These observations support the possibility that the nutritive value of milk from mothers with SCM may be altered by asymptomatic breast inflammation. Collectively, these findings provide evidence that SCM for several reasons may be an

TABLE 3 Multiple logistic regression models for infant stunting (HAZ < -2SD), underweight (WAZ < -2SD), and for low head circumference (HCAZ < -2SD)^a

	Odds ratio (OR)	95% CI	P value
Model 1: Stunting ^b			
Subclinical mastitis, yes	4.28	[1.15, 15.84]	0.029
Lactation stage (established)	2.34	[0.95, 5.77]	0.065
Maternal height, cm	0.88	[0.79, 0.96]	0.007
Infant diarrhoea, yes	7.07	[0.64, 37.25]	0.108
Maternal stool <i>Entamoeba coli</i> , yes	0.89	[0.36, 2.18]	0.799
Model 2: Underweight ^c			
Subclinical mastitis, yes	9.21	[1.76, 48.00]	0.008
Lactation stage (early)	1.25	[0.35, 4.50]	0.732
Feeding frequency, #/day	0.78	[0.59, 1.04]	0.087
Maternal stool <i>Entamoeba coli</i> , yes	0.78	[0.22, 2.75]	0.695
Model 3: Low head circumference ^d			
Subclinical mastitis, yes	15.89	[2.61, 96.86]	0.003
Lactation stage (early)	0.43	[0.13, 1.43]	0.167
Maternal age at first pregnancy, years	0.89	[0.70, 1.14]	0.359
Feeding frequency, #/day	0.74	[0.56, 0.97]	0.031
Maternal stool <i>Entamoeba coli</i> , yes	3.28	[1.02, 10.58]	0.047

Note. HAZ: height-for-age Z score; HCAZ: head circumference-for-age z-score; WAZ: weight-for-age Z score; SD: standard deviation; CI: confidence interval.

^aThe variable "lactation" stage, defined as early (0–6 weeks) or established (4–6 months) lactation, controlled for timing of milk collection as well as composition differences between the collection periods.

^bOverall model $\chi^2(5) = 16.54$, Nagelkerke $R^2 = 0.208$, $P = 0.005$; Hosmer and Lemeshow $\chi^2(8) = 6.81$, $P = 0.557$. For stunting, univariate variables ($P \leq 0.25$) included in the initial model were maternal (cough and stomach ache) and infant health (cough, and diarrhoea) and maternal intestinal protozoa (*Blastocystis hominis*, *Endolimax nana*, and *Entamoeba coli*) in addition to SCM and lactation stage.

^cOverall model $\chi^2(4) = 8.43$, Nagelkerke $R^2 = 0.151$, $P = 0.077$. The Hosmer and Lemeshow $\chi^2(8) = 9.80$, $P = 0.279$. For underweight, univariate variables ($P \leq 0.25$) included in the initial model were maternal age < 34 years, *Entamoeba coli* and breastfeeding frequency (#/day) in addition to SCM and lactation stage.

^dOverall model $\chi^2(5) = 18.45$, Nagelkerke $R^2 = 0.248$, $P = 0.002$. The Hosmer and Lemeshow $\chi^2(8) = 13.23$, $P = 0.104$. For low head circumference, univariate variables ($P \leq 0.25$) included in the initial model were maternal age at first pregnancy, height, weight, and body mass index, maternal health (headache), maternal intestinal protozoa (*Entamoeba coli*), and breastfeeding frequency (#/day, #/24 hr) in addition to SCM and lactation stage.

overlooked maternal factor that underscores growth faltering in breastfed infants.

Subclinical mastitis and its potential impact on the composition and nutritive value of breastmilk is only one factor possibly associated with early infant growth. Poor hygiene and sanitation can contribute to environmental enteropathy (Keusch et al., 2013) and to infant diarrhoea, which is considered to be the most important contributor to stunting in children less than 2 years in Latin America (Mosites et al., 2017). Consistent with this, water, sanitation and hygiene (WASH) interventions often (Cumming & Cairncross, 2016; Tofail et al., 2018), but not always (Null et al., 2018; Stewart et al., 2018), reduce diarrhoea and improve weight and/or length. In our communities, we

have evidence of poor sanitation as only 15% of households had a toilet and of poor maternal hygiene and faecal–oral contamination as 70% of mothers had at least one species of nonpathogenic intestinal protozoa and 25% of mothers reported that their infant had diarrhoea in the previous week. Yet neither our indicators of hygiene (latrine, faucet, faecal–oral contamination) nor infant diarrhoea emerged in models for stunting or for underweight. In contrast, however, the odds of low head circumference were higher if maternal stool samples contained *Entamoeba coli*. This nonpathogenic protozoan is transmitted through the faecal–oral route indicating an unhygienic environment. Furthermore, nonpathogenic and commensal microbes in infant stool, independent of diarrheal diseases, have been linked with environmental enteric dysentery and growth stunting in children (Harper, Mutasa, Prendergast, Humphrey, & Manges, 2018; Mbuya & Humphrey, 2016). However, there is very little available information that could explain a direct link between maternal *Entamoeba coli* and low infant head circumference. A recent laboratory study reported that an intestinal infection of pregnant mice impaired fetal brain gene expression (Haque, Starr, Koski, & Scott, 2018), but such a direct link with low head circumference has not been reported in humans. Thus, at best, the relationship may be indirect in that *Entamoeba coli* may be a bio-marker associated with other maternal faecal pathogens or health status.

The importance of breast milk for infant nutrition, growth, and development is well established (Neville et al., 2012), but less is known about the impact of optimal breastfeeding practices on infant growth (Jones et al., 2014). It is recommended that breastfeeding be initiated within 1 hr of birth and that mothers exclusively or predominantly breastfeed prior to 6 months and breastfeed at least 8–12 times every 24 hr (WHO, 2003). A meta-analysis reported that lack of compliance with the WHO recommendation for exclusive breastfeeding to 6 months of age did not impact infant WAZ, LAZ, or WLZ (Kramer & Kakuma, 2012), although a recent study found a weak benefit for following this recommendation for WAZ and WLZ, but not for LAZ (Eriksen et al., 2017). It has also been shown that initiation of breastfeeding within 1 hr post-partum was positively associated with breastfeeding frequency and that higher breastfeeding frequency has been associated with a higher WHZ, but not with WAZ, LAZ, or HCAZ in Guatemalan infants (Wren et al., 2015). One previous study had observed that infant weight and breastfeeding frequency were independently associated with breast milk intake and that the effect of SCM on milk volume disappeared if both infant weight and feeding frequency were entered in multiple linear regression models (Aryeetey et al., 2009). Despite inclusion of both variables in our study, SCM remained in our models and was associated with the highest attributable risk factor of SCM with low head circumference. One study had associated a similar SCM ratio in early lactation with 18% of mothers in the United States stopping breastfeeding by day 60 post-partum (Murase, Wagner, Chantry, Dewey, & Nommsen-Rivers, 2017). However, in our population, mothers did not stop breastfeeding by 2 months post-partum and regularly used a sauna (e.g., temescal) several times per week to avoid milk stasis and maintain milk flow; this cultural practice used by Mam-Mayan mothers has been associated with higher breastfeeding frequency (Wren et al., 2015). Higher breastfeeding frequency, in turn, has been associated with increased provision of nutritive, trophic, and bioactive factors to promote early

post-partum brain development (Neville et al., 2012; Victora et al., 2016) and may underscore its beneficial effect on head circumference in our study.

4.1 | Limitations

Several limitations are present. Inferring causality is precluded by the cross-sectional design. Others had reported in older infants that it was difficult to determine if higher breastfeeding frequency or higher infant weight were driving milk intakes (Kumwenda et al., 2016) as is the case with this study. By collecting unilateral breast milk samples, we may have underestimated the prevalence of SCM, although one paper reported less than 5% discordance in SCM between breasts (Kasonka et al., 2006). Only maternal and not infant stool samples were collected, and episodes of infant diarrhoea as well as breastfeeding frequency may have been subject to recall bias. It is possible that the association between maternal *Entamoeba coli* and infant head circumference may be a chance statistical finding and may be a non-specific proxy for an unsanitary environment.

5 | CONCLUSION

Reducing growth faltering in breastfed infants prior to 6 months of age has been a challenge in part because recognition of the primary drivers of infant growth faltering is incomplete (Eriksen et al., 2018; Mosites et al., 2017). Our findings provide evidence that two maternal health conditions, SCM and maternal faecal-oral contamination, may play a more prominent role in early infant growth faltering than previously recognized and that promotion of higher breastfeeding frequency may reduce the risk of low head circumference, at least in populations where growth faltering is of concern. These findings expand the paradigm for early infant growth faltering from a focus on dietary interventions and preventing infant diarrhoea to include maternal health status and the contribution of SCM, hygiene, and early breastfeeding practices to growth of breast fed infants.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

CONTRIBUTIONS

All authors were involved in conceptualization and design of the study. HMW-A conducted the field research, analysed the data, and wrote the first draft of the manuscript. All authors provided input into the final manuscript.

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