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Enteroaggregative *E. coli* subclinical infection and co-infections and impaired child growth in the MAL-ED cohort study

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Abstract

Objective: We evaluated the impact of subclinical enteroaggregative *Escherichia coli* (EAEC) infection alone and in combination with other pathogens in the first six months of life on child growth.

Methods: Non-diarrheal samples from 1,684 children across eight Multisite Birth Cohort Study, Malnutrition and Enteric Diseases (MAL-ED) sites in Asia, Africa, and Latin America were tested monthly; over 90% of children were followed-up twice weekly for the first six months of life.

Results: Children with subclinical EAEC infection did not show altered growth between enrollment and six months. Conversely, EAEC co-infection with any other pathogen was negatively associated with delta weight-for-length (WLZ) ($p < 0.05$) and weight-for-age (WAZ) ($p > 0.05$) z-scores between 0 and 6 months. The presence of two or more pathogens without EAEC was not significantly associated with delta WLZ and WAZ. The most frequent EAEC co-infections included *Campylobacter spp.* heat-labile toxin-producing enterotoxigenic *E. coli, Cryptosporidium spp.*, and atypical enteropathogenic *E. coli*. Myeloperoxidase levels were increased with EAEC co-infection ($p < 0.05$). EAEC pathogen co-detection was associated with lower neopterin levels compared to those of no-pathogen control children ($p < 0.05$). Mothers of children with EAEC co-infections had lower levels of education, poorer hygiene and sanitation, lower socioeconomic status, and lower breastfeeding rates compared to mothers of children in whom no pathogen was detected ($p < 0.05$).

Conclusions: These data emphasize the public health importance of subclinical EAEC infection in early infancy in association with other pathogens and the need for improved maternal and child care, hygiene, sanitation, and socioeconomic factors.

Keywords: Enteroaggregative *Escherichia coli*, pathogen EAEC co-infection, gut inflammation, intestinal immune responses, nutritional status.

Summary box:

What is known?

- In pediatric cohort studies, enteroaggregative *Escherichia coli* (EAEC) was frequently detected in developing countries;
- EAEC was associated with growth deficits in these children;
- The potential impact of EAEC subclinical infections and the presence of co-infections on child growth remains unclear.

What is new?

- Isolated subclinical EAEC infection does not influence child growth in the first six months of life;
- Increased pathogen EAEC co-infection is negatively associated with delta weight-for-length and weight-for-age z-scores, which are partially dependent on the presence of EAEC.

These data emphasize the importance of subclinical EAEC co-infections in early childhood.

Introduction

Escherichia coli are an important cause of enteric infections, and the following five enterovirulent types have been identified: (a) enterotoxigenic *E. coli* (ETEC), (b) Shiga toxin-producing *E. coli* (STEC), (c) enteroinvasive *E. coli* (EIEC), (d) enteropathogenic *E. coli* (EPEC), and (e) enteroaggregative *E. coli* (EAEC) (1-3). The multisite birth cohort study, Malnutrition and Enteric Diseases (MAL-ED), involved intensive community surveillance for diarrhea and non-diarrheal stools over the first two years of life from eight sites in South America, Africa, and Asia, showed that EAEC were frequently detected in children with and without diarrhea (4). These results were consistent with early data reported in a small cohort study in Fortaleza CE, Brazil (5). The EAEC pathogen burden in the MAL-ED birth cohort study was associated with growth deficits in these children (6). However, the potential impact of EAEC associated with co-infections in early infancy on child growth remains unclear. The MAL-ED birth cohort study also reported that both the incidence of diarrhea and the number of pathogens detected per stool increased markedly during the first year of life (4). Two or more pathogens were identified in 41% (2,999) of diarrhea samples and 29% (7,046) of non-diarrheal samples, suggesting that co-infections are common in these children in their first year of life. Therefore, we examined subclinical or "silent" EAEC infections alone and in combination with other pathogens and their associations with child growth in the first six months of life.

Early studies have shown that EAEC is an inflammatory pathogen and that growth deficits occur in children when the bacterium is acquired, with or without diarrheal symptoms (7,8). The potential impact of subclinical EAEC infections and the presence of co-infections on the pathobiology of EAEC infections and effects on child growth remain unclear.

We hypothesized that maternal education, birth weight, breastfeeding, and socioeconomic status will increase the risk of acquiring asymptomatic EAEC alone or with other enteric

pathogens in early infancy, leading to gut inflammation and impaired growth. The aim of this study was thus to understand the risk factors, gut integrity, inflammation, and innate immune responses associated with EAEC or in combination with other enteric pathogens and their impact on growth in the first six months of life across all eight sites in the MAL-ED multisite birth cohort study.

Methods

Study setting

This study was conducted across eight locations: Dhaka, Bangladesh (BGD); Fortaleza, Brazil (BRF); Vellore, India (INV); Bhaktapur, Nepal (NEB); Loreto, Peru (PEL); Naushero Feroze, Pakistan (PKN); Venda, South Africa (SAV); and Haydom, Tanzania (TZH). A detailed description of the MAL-ED study location, demography, and socio-economic status has been reported elsewhere (9-16).

Study design, population, and ethical approval

In this longitudinal birth cohort study, infants up to two years of age were followed-up in each of the eight study sites; this report included data collected during the first six months of life. The overall design of the project has been described in detail elsewhere (17-24). The study and consent protocols were approved by the local institutional review board (IRB) at all sites as well as the collaborating institution IRBs. Written informed consent was obtained from the parent or guardian of every child. We enrolled infants within 17 days of birth between November 2009 and February 2012.

Surveillance and stool collection

Surveillance was performed during twice-weekly household visits. Caregivers responded to a standardized questionnaire designed to collect data regarding daily symptoms of cough,

fever, vomiting, diarrhea, and medication use. We investigated non-diarrheal specimens that were collected during the surveillance between one and six months of age. The overall surveillance methods utilized in the MAL-ED cohorts have been described in detail elsewhere (17).

Maternal education, birthweight, and breastfeeding variables, and socio-economic status

Questionnaires were developed to collect information about child anthropometrics, child care, characteristics of the mother or caregiver, household, people usually sleeping in the house, sources of water, toilet facilities, average monthly income, and other related parameters. For socioeconomic status (SES) determination, we used a standardized SES questionnaire applicable to the MAL-ED cohorts (25). The defined variables and their parameterizations are shown in **Supplementary Table 1 (Supplemental Digital Content 1, http://links.lww.com/MPG/B92)**.

Anthropometric measurements

The study protocol used a standard recumbent length measuring board (Schorr Productions, Olney, MD) to measure the monthly length of all enrolled children to the nearest 0.1 cm. Digital scales were also used monthly to measure weight to the nearest 100 g. The weight-for-age (WAZ), length-for-age (LAZ), and weight-for-length (WLZ) z-scores were calculated using the World Health Organization Multi-Country Growth Reference Study (26).

Microbiology and stool testing

The non-diarrheal specimens were analyzed in accordance with a standardized microbiology protocol, which was implemented at all study sites. The protocol has previously been described in detail (21). Briefly, we selected a pool of five lactose-fermenting colonies resembling *E. coli* and characterized them for virulence genes using a multiplex polymerase chain reaction (PCR) assay. Details of the virulence genes selected for the PCR probes are presented in the references of the paper by Houpt et al. (21).

Gut function integrity, immune and inflammatory biomarkers

The lactulose:mannitol test was used to evaluate intestinal permeability, malabsorption, and damage and was administered to children at three and six months. The average of these two measurements used for the analysis. Lactulose and mannitol were measured as previously described (27).

Three additional biomarkers were also measured monthly in non-diarrheal stools between 0 and 6 months of age and the average of these measurements used for this analysis. These included alpha-1-antitrypsin (A1AT), myeloperoxidase (MPO), and neopterin (NEO) (27).

The MAL-ED cohort study used a standardized protocol and data collection tools (28). On-site training, quality assurance, and quality control protocols enabled this study to maintain a harmonized, quality database for analysis. The data were entered using a double data entry system in Microsoft Access (Microsoft Corporation, Redmond, Washington).

Statistical analysis

We evaluated cumulative EAEC infection alone and in combination with any other enteric pathogen in monthly non-diarrheal stools from asymptomatic children across eight MAL-ED sites in Asia, Africa, and Latin America. Children were selected when they had ≥90% active surveillance (Surveillance Assessment Form) for 0-6 months, had collected a Follow-up Socio-Economic status form for 0-6 months, and had stool samples with a complete microbiology workup.

Pathogen co-infections and outcome variables

To determine the impact of EAEC infection alone and in combination with any other enteric pathogen co-infection, we divided the cohort children into seven groups based on the cumulative monthly stool detection of enteric pathogens as follows: (1) children with no pathogen detection in every stool collected; (2) children with EAEC in any stool collected; (3) children with EAEC and one other pathogen; (4) children with EAEC and two other pathogens; (5)

children with EAEC and three or more other pathogens; (6) children with one or two pathogens other than EAEC; and (7) children with three or more pathogens other than EAEC.

The major outcome variables were weight-for-age, length-for-age, and weight-for-length deltas z-scores (0-6 months). The secondary outcome variables included the average 0-6 months' gut function, inflammation, and innate immune response marker association in the study groups. Categorization of groups based on cumulative EAEC infection alone or co-infection with any other enteric pathogen enabled us to assess the impact of EAEC alone or in combination with other enteric pathogens on child growth.

Chi-square or Fisher exact tests were performed to compare categorical variables between the seven groups defined above. Student's *t*-tests for normally distributed data and Kruskal-Wallis tests for non-normally distributed data were used to compare continuous variables between these groups.

We performed a mixed-effects linear regression analysis on child growth in the first six months of life. The differences in WAZ, WLZ, and LAZ z-scores between enrollment and 6 months were regressed against EAEC exposure with and without other enteric pathogens. We included model covariates based on biologic plausibility that included child sex, weight and/or length at enrollment, an indicator of household food insecurity, and proportion of days of breastfeeding, symptoms of acute lower respiratory infection, and antibiotic use. Definitions of the surveillance covariates are provided in **Supplementary Table 1 (Supplemental Digital Content 1, http://links.lww.com/MPG/B92)**. The model included a random intercept on the study site to account for unexplained variation in the model due to site variability.

Statistical analysis was conducted using IBM SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY). Regression and plots were performed in R v.3.2.2 using the 'lme4' package. *P* values < 0.05 were considered statistically significant.

Results

Participant enrollment and selection. Across eight study sites, 2,145 children were enrolled on a rolling basis between November 2009 and February 2012 and longitudinally followed. Of these, stool samples with a complete microbiology workup were available for 1,695 children. Finally, 1,684 children who underwent a minimum of 90% of the active surveillance assessments between 0 and 6 months and whose parents or legal guardians had answered a socio-economic status questionnaire during this time were included in the final analysis. The 1,684 children provided 8,216 surveillance stools up to six months of age. The groups of children and details of the stools provided for analysis are shown in **Table 1**.

Association of potential risk determinants with subclinical EAEC infection alone or in combination with other pathogens. **Table 1** summarizes the categorical risk variables associated with subclinical EAEC infections through the first six months of age. There were no differences in sex or age between the groups tested. The proportions of children with birth weights less than 2,500 g were also similar among all groups. Mothers with less than six years of schooling and no suitable drinking water sources were significantly more common for children with subclinical EAEC infections with ≥3 other pathogens compared to all other groups of children. Both subclinical infections with EAEC plus ≥3 other pathogens and ≥3 pathogens without EAEC were significantly more common in children with inadequate sanitation compared to the other pathogen groups. Food insecurity was present in a smaller proportion in the group of children with subclinical EAEC infection alone compared to no pathogens. Children with subclinical EAEC infection with only one other pathogen had less food insecurity compared to EAEC with two other pathogens. **Table 2** shows the quantitative risk variables associated with subclinical EAEC infections through the first six months of age. Birthweights, mothers' years of schooling, and monthly incomes were similar across all groups of children. The SES measured via the *asset*

score was lower in the group with EAEC combined with ≥3 other pathogens compared to the scores for all other groups of children. The SES was also lower in the group with EAEC combined with one or two other pathogens compared to those in the following groups: no pathogen, EAEC alone, and 1 or 2 pathogens without EAEC. Finally, the SES was lower in the group with ≥3 pathogens without EAEC than in the non-pathogens group. A similar significant trend was also observed for SES measured via the water/sanitation, household assets, maternal education, and household income (*WAMI*) as shown in **Table 2**. The proportion of days of antibiotic use was significantly higher in the group of children with EAEC subclinical infection with ≥3 other pathogens than in all other groups. The proportion of breastfeeding days was significantly lower in the group with EAEC subclinical infections with ≥3 other pathogens compared to those in all other groups except for ≥3 pathogens without EAEC. The proportion of breastfeeding days was also lower in the group with EAEC combined with two other pathogens compared to that in the non-pathogen group.

Gut function barrier integrity, and immune and inflammatory biomarkers. Gut function as measured by the adjusted z-scores of the percentages of lactulose and mannitol excreted in the urine and lactulose:mannitol ratios were similar across all groups of children (**Table 3**). Stool myeloperoxidase (MPO) concentrations were significantly higher in the subclinical EAEC infection with ≥3 other pathogens group than that in the non-pathogen group. MPO concentration was also higher in children with EAEC with two other pathogens than in the non-pathogen group. Lower concentrations of stool neopterin were found in the groups of children with subclinical EAEC infection with ≥3 other pathogens compared to all other groups of children, except ≥3 pathogens without EAEC; children with EAEC with two other pathogens (vs. no pathogens and vs. 1 or 2 pathogens without EAEC); children with ≥3 pathogens without EAEC (vs. no pathogens and vs. 1 or 2 pathogens without EAEC). Stool alpha-1-antitrypsin marker concentrations were comparable across all groups of children.

Effect of subclinical EAEC infections alone or combined with any other pathogen on cumulative child growth. Cumulative growth was measured up to six months of age. **Table 4** and **Supplementary Figure 1 (Supplemental Digital Content 2, http://links.lww.com/MPG/B93)** summarize the delta z-scores WLZ, WAZ, and LAZ. Children with EAEC with ≥3 other pathogens showed an impaired delta WLZ z-score compared to all other groups, except for EAEC with two other pathogens. The delta WAZ z-score was also significantly lower in the EAEC with ≥3 other pathogens group compared to all other groups, except for EAEC with two other pathogens and non-pathogens (borderline; p = 0.056).

The regression results of the association between EAEC exposure, in the absence of other pathogens, with growth over the first six months of life were not statistically significant (**Figure 1**). However, when EAEC was associated with co-infection with one, two, or three additional pathogens, decreased delta WLZ and WAZ were observed (**Figure 1**). When EAEC was associated with co-infection with three or more additional pathogens, weight-for-length was -0.244 z-scores lower than that in children with no pathogens present (p <0.05, **Figure 1**). This association was not maintained when three or more pathogens were present in the absence of EAEC (**Figure 1**).

Prevalence of subclinical enteric co-infections with EAEC. Across the cohort's study sites, we observed that the groups of children with subclinical EAEC infection with two or ≥3 other pathogens were strongly associated with impaired physical growth, as measured by WLZ and WAZ delta z-scores. Therefore, we explored the prevalence of subclinical enteric co-infections with EAEC in these two groups of children. **Figure 2A** shows that the highest prevalence of enteric co-infection involved *Campylobacter* spp. and atypical enteropathogenic *Escherichia coli* in children with EAEC and two pathogens. *Campylobacter* spp., enterotoxigenic *E. coli* thermo-labile toxin (LT)-producing, and *Cryptosporidium* spp. were the most prevalent co-infection

pathogens in children with EAEC with ≥3 other pathogens. **Figure 2B** shows only the enteric pathogens with more than 1% of the cumulative prevalence up to six months of age.

Discussion

In non-diarrheal stool samples, subclinical EAEC infection alone was not significantly associated with child growth between enrollment and six months of age. However, increasing pathogen co-detection with EAEC was negatively associated with decreased delta WAZ and WLZ from 0-6 months. Compared to children with no pathogens detected, the mean delta WLZ was approximately 0.25 lower in children with EAEC and three or more pathogens ($p < 0.05$) and the mean delta WAZ was approximately 0.16 lower in children with EAEC and three or more pathogens ($p > 0.05$). There was no clear trend in the correlation between EAEC exposure and LAZ with or without pathogen co-detection. However, early cohort studies reported an association between EAEC subclinical infection and child growth impairment (6,7). A previous analysis of EAEC in the MAL-ED study showed that consistent detection of EAEC across the first two years of life was associated with linear growth deficits at two years of age (Rogawski et al., manuscript under review). However, this previous work did not explore co-infection. In addition, increasing pathogen EAEC co-detection was negatively associated with WLZ and WAZ in the present study. This effect was not observed in other pathogen co-infections in the absence of EAEC. These results suggest a pathobiological interaction between EAEC and other pathogens, resulting in growth impairment at a critical stage of early development. A recent study using microbiota from a Malawian birth cohort in an undernourished donor community administered to recipient gnotobiotic mice produced a growth deficit compared to that from a healthy donor community (29). These data suggest the hypothesis that gut microbiota immaturity together with enteric co-pathogens including EAEC may impact growth development or have other long-term consequences, a possibility that warrants further study.

Few studies have evaluated isolated EAEC subclinical infection with determinant variables; several have reported an association between inadequate or contaminated food and water and EAEC infection without examining pathogen co-detection (30-32). Comparable associations between EAEC infection and poor hygiene and host immunosuppression have been reported (33). Therefore, we limited the focus of this work to the variables associated with EAEC co-infections in the current literature.

Consistent with recent publications from the MAL-ED birth cohort and BRF site case-control MAL-ED studies, EAEC alone and all the other groups of children presented with high urine LM-Z ratio and fecal MPO, A1AT, and NEO biomarker levels, suggesting environmental enteropathy disease (27,34). MPO concentration was significantly higher in children with EAEC and three or more pathogens and in children with EAEC with co-detection of two other pathogens compared to the concentrations in children in which no pathogens were detected. These results suggest an interaction effect of EAEC with two or more pathogens on gut inflammatory responses but not in the absence of EAEC with two or more pathogens. Studies have shown colonization of EAEC without overt symptoms of diarrhea; however, these studies did not evaluate pathogen co-detection (7,8). Furthermore, the studies concluded that EAEC was an inflammatory pathogen and that infection resulted in growth deficit even without overt symptoms of diarrhea (7,8). The present report suggests for the first time that early childhood growth deficits are associated with EAEC and co-infections in an asymptomatic cohort of children. The pathobiology of subclinical EAEC infection with pathogen co-detection appears to be more complex than previously thought; therefore, further studies are required to examine this in detail. NEO concentration was higher in the non-pathogen group compared to that in EAEC group with two or more other pathogens and in the group with three or more pathogen co-detection without EAEC. These data suggest greater protection in the gut immune responses in the control group compared to the other groups of children.

This study has several limitations. First, despite monthly stool analysis over six months, subclinical pathogen co-detection could have been missed between stool sample collections. Second, the study group definition and design analyses do not account for the duration of subclinical infections, even though we had a sense of repeated detection and quantitative specific pathogen detection. These are relevant parameters to consider for further studies on the pathobiology and impact of isolated subclinical EAEC and co-detected pathogen infections on physical growth in children. The study also has several strengths. First, the MAL-ED multicenter study collects comprehensive information on determinant variables such as maternal and child care characteristics, sanitation, hygiene, and SES, which enable assessment of the association or influence of the determinant variables on child nutritional status after six months of follow-up. Second, the study also collects information on important biomarkers, allowing for examination of key potential pathobiological association with the subclinical isolated EAEC and co-infections. Third, the MAL-ED study allowed co-infection analysis to examine the potential interaction associations of isolated EAEC and pathogen co-infection with child growth.

In conclusion, silent (i.e., acutely asymptomatic) enteric co-infections with EAEC and other pathogens in the first six months of life were associated with significant growth deficits (decreasing delta WLZ z-score). Further study of the potential associations and mechanisms of infection with EAEC alone and co-infection with other potential pathogens is warranted, and potential strategies to prevent growth deficit or other long-term consequences are required.

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Figure 1: Changes in weight-for-length (WLZ) z-scores from 0 to 6 months of age (delta $WLZ_{0\text{-}6m}$) by EAEC with or without copathogens when compared with the delta $WLZ_{0\text{-}6m}$ in children with no enteric pathogen detected over 0-6 months of age. Coefficients refer to the number of standard deviations a dependent variable (children with no pathogen group) will change, per standard deviation increase in the predictor variable (all other groups of children, see figure). This figure shows the beta coefficient estimate for delta WLZ. Note that when EAEC is present with one, two, or three additional pathogens, there is a progressive decrease in the delta WLZ. This relationship is not maintained when three or more pathogens are present in the absence of EAEC.

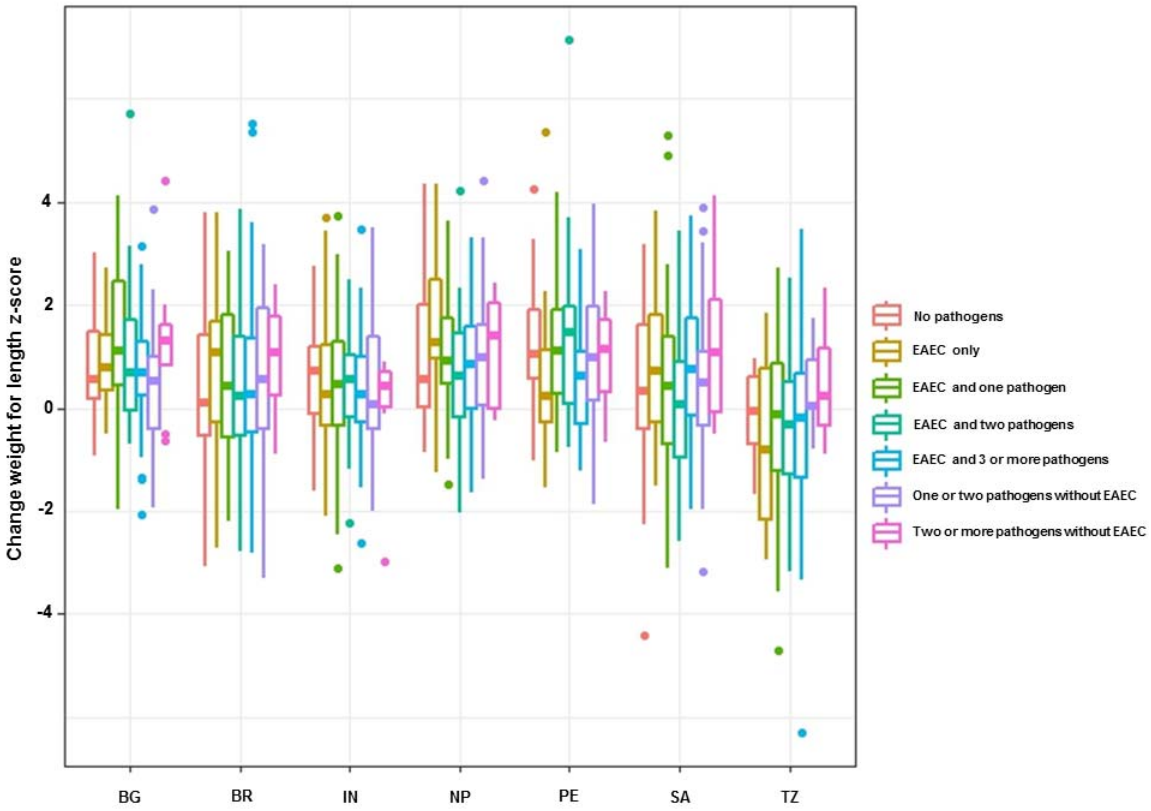
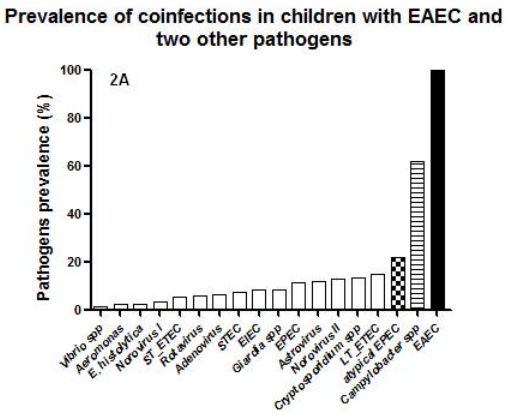


Figure 2: Prevalence of specific pathogens in children with enteroaggregative *Escherichia coli* (EAEC) with two (**Figure 2A**) and three or more (**Figure 2B**) pathogen co-infections, respectively, in monthly stool samples in the first six months of life. EAEC=enteroaggregative *Escherichia coli*; EIEC=enteroinvasive *E. coli*; aEPEC=atypical enteropathogenic *E. coli*; tEPEC=typical enteropathogenic *E. coli*; LT/ST-ETEC=LT/ST-producing enterotoxigenic *E. coli*; STEC=Shiga-toxin-producing *E. coli*. Pathogens present in less than 1% of stool samples are not shown.



Prevalence of coinfections in children with EAEC and ≥ 3 other pathogens

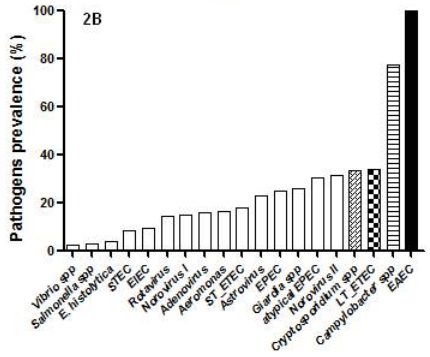


Table 1 - Determinant categorical variables associated with EAEC carriage over 0-6 months of age.

Determinant variables			Groups of children						
			No pathogens	EAEC_P0	EAEC_P1	EAEC_P2	EAEC_P3	1 or 2 Pathogens without EAEC	≥3 Pathogens without EAEC
Number of children	**Total**	1,684	171	198	321	292	363	266	73
Surveillance stools contributed	**Total**	8,216	708	923	1584	1459	1915	1277	350
Gender	**Male**	Count	82_a	94_a	169_a	162_a	176_a	142_a	33_a
		%	48.0%	47.5%	52.6%	55.5%	48.5%	53.4%	45.2%
Total		Count	171	198	321	292	363	266	73
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Birth weight <2500 g	**yes**	Count	15_a	20_a	36_a	26_a	21_a	23_a	6_a
		%	9.1%	11.2%	12.8%	10.7%	9.7%	9.4%	9,7%

Total		Count	165	179	281	243	216	245	62
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Suitable source of drinking water	**no**	Count	7_{a}	$15_{a.\ b.\ c.\ d}$	$30_{c.\ d}$	$30_{b.\ d}$	73_{e}	$15_{a.\ b.\ c.\ d}$	$4_{a,\ b,\ c,\ d}$
		%	4.3%	8.1%	9.8%	10.6%	20.7%	6.1%	5,6%
Total		Count	161	186	306	282	352	246	71
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Suitable sanitation	**no**	Count	43_{a}	50_{a}	84_{a}	93_{a}	147_{b}	74_{a}	$21_{a,\ b}$
		%	26.7%	26.9%	27.5%	33.0%	41.8%	30.0%	29,6%
Total		Count	161	186	306	282	352	247	71
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Mother with <6yrs of schooling	**yes**	Count	34_{a}	$56_{a.\ b}$	$108_{b.\ c.\ d}$	111_{d}	185_{e}	$69_{a.\ b}$	$32_{c,\ d,\ e}$
		%	21.1%	30.1%	35.4%	39.5%	52.6%	27.9%	45,1%
Total		Count	161	186	305	281	352	247	71
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Food Insecurity	**yes**	Count	$76_{b.\ c}$	66_{a}	$122_{a.\ b}$	135_{c}	$155_{a.\ b.\ c}$	127_{c}	$34_{b,\ c}$
		%	49.7%	37.5%	41.4%	49.6%	45.5%	53.4%	52,3%
Total		Count	153	176	295	272	341	238	65
		%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Each subscript letter denotes a subset of groups of children categories whose column proportions do not differ significantly from each other at the 0.05 level (Chi-Square tests).

* Enteroaggregative *E, coli* (EAEC) with none (P0), one (P1), two (P2), and three (P3) or more any other pathogens.

Table 2 - Determinant quantitative variables associated with EAEC carriage over 0-6 months of age.

Determinant variables		Groups of children						
		No pathogens	EAEC_P0	EAEC_P1	EAEC_P2	EAEC_P3	1 or 2 Pathogens without EAEC	≥3 Pathogens without EAEC
Birthweight (Kg)	Median	3.05	3.00	3.00	3.05	3.00	3.09	3.00
	Minimum	1.90	1.80	1.69	1.78	1.80	1.90	2.25
	Maximum	4.90	4.10	4.27	4.50	4.60	4.75	4.00
Mother schooling (years)	Median	9	8	7	7	5	8	6
	Minimum	0	0	0	0	0	0	0
	Maximum	17	18	16	15	15	17	13
Income (US$/Month)	Median	115.00	117.50	113.05	104.55	103.50	136.50	123.70
	Minimum	3.0	1.2	2.4	3.6	1.8	6.0	7.0
	Maximum	966.7	625.2	1056.0	924.0	994.0	1648.4	812.0
Asset	Median	6.00	5.00	4.00§	4.00**	3.00*	5.00	4.00†

	Minimum	0	0	0	0	0	0	0
	Maximum	8	8	8	8	8	8	8
WAMI	Median	.625	.617	.563§	.547**	.438*	.609	.563
	Minimum	.031	.063	.000	.000	.000	.000	.031
	Maximum	.953	1.000	.984	.953	.953	1.000	.953
% of days with acute lower respiratory infections	Median	1.1	.0	2.2	.0	.0	2.2	.0
	Minimum	.0	.0	.0	.0	.0	.0	.0
	Maximum	6.6	9.2	7.6	5.9	7.7	7.0	9.2
% of days using antibiotics	Median	2.7	2.7	3.8	2.9	6.0*	2.7	2.7
	Minimum	.0	.0	.0	.0	.0	.0	.0
	Maximum	42.4	47.3	52.5	75.1	61.1	53.8	50.0
% of days with breastfeeding	Median	98.8	98.4	98.4	98.3**	96.8*	98.4	97.8
	Minimum	58.1	.0	.0	.0	.0	.0	44.9
	Maximum	100.0	100.0	100.0	100.0	100.0	100.0	100.0
% of days with	Median	6.8	9.2	4.4	3.0**	7.1	9.2	4.9

dehydration	Minimum	2.2	2.8	1.1	.5	.5	1.1	.5
	Maximum	11.4	24.5	63.6	23.6	40.8	38.0	20.2
% of days with	Median	.6	.6	1.1	1.1	1.6	1.6	1.7
diarrhea	Minimum	.0	.0	.0	.0	.0	.0	.0
	Maximum	89.7	53.3	67.4	43.7	49.2	54.9	37.3
% of days with	Median	.0	.0	.0	.0	.0	.0	.0
fever	Minimum	.0	.0	.0	.0	.0	.0	.0
	Maximum	8. 7	18.4	47.8	5.0	18.9	20. 6	12.6

Asset (sum of 8 scores; has mattress, chair, table, tv, refrigerator, bank account, kitchen, <2 people per room) pairwise comparisons by independent-samples Kruskal-Wallis Test: * EAEC_P3 vs all other groups ($p< 0.05$); ** EAEC_P2 vs non-pathogens, EAEC_P0 and 1 or 2 pathogens without EAEC ($p<0.05$); § EAEC_P1 vs non-pathogens, 1 or 2 pathogens without EAEC ($p<0.05$); and † ≥3 pathogens without EAEC vs non-pathogens ($p<0.05$).

Wami (Sum of the following variables: sanitation, asset, incat, newfseschool and dividing the total by 32): * EAEC_P3 vs all other groups except ≥3 pathogens without EAEC ($p< 0.05$); ** EAEC_P2 vs Non-pathogens and 1 or 2 pathogens without EAEC ($p<0.05$); § EAEC_P1 vs non-pathogens ($p<0.05$).

% of days using antibiotics: * EAEC_P3 vs all other groups except ≥3 pathogens without EAEC ($p< 0.05$);

% of days with breastfeeding: * EAEC_P3 vs all other groups except ≥3 pathogens without EAEC ($p < 0.05$); ** EAEC_P2 vs non-pathogens ($p<0.05$).

% of days with dehydration: ** EAEC_P2 vs 1 or 2 pathogens without EAEC ($p<0.05$).

Table 3 – Biomarkers associated with no pathogens or with EAEC carriage and co-infections with other enteric pathogens.

Biomarkers		Groups of children						
		Non pathogens	EAEC_P0*	EAEC_P1	EAEC_P2	EAEC_P3	1 or 2 Pathogens without EAEC	≥3 Pathogens without EAEC
Z-score %lactulose	Median	.9343	.8767	.9832	.7951	.8696	.9417	1.0582
	Minimum	-1.9429	-2.0421	-2.1568	-3.2737	-4.5702	-2.2246	-2.3525
	Maximum	7.1715	4.9319	6.4300	7.9253	6.4066	5.3051	3.9377
Z-score %mannitol	Median	.4036	.4575	.3923	.3852	.3478	.4771	.4711
	Minimum	-1.4403	-2.1661	-1.9402	-2.6062	-2.1725	-1.8277	-1.9379
	Maximum	2.6551	3.1492	2.6375	3.9473	3.9912	2.4371	2.4148
Z-score lactulose	Median	.2370	.2509	.3106	.2235	.3003	.2099	.1884

/ mannitol ratio	Minimum	-1.6894	-6.9774	-2.3467	-6.1037	-2.9995	-2.9296	-2.4921
	Maximum	2.2237	2.4843	1.9751	2.8774	2.1294	1.8635	1.8433
Myeloperoxidase (ng/mL)	Median	5877.25	7447.02	7446.47	8290.56**	9039.79*	7480.78	8375.42
	Minimum	634.99	167.88	1045.33	452.45	257.86	349.00	660.42
	Maximum	44025.94	73561.05	66145.51	58975.13	46209.77	49072.82	28763.12
Neopterin (nmol/L)	Median	2298.41	2229.06	2045.85	1760.84**	1300.00*	2176.95	1578.38§
	Minimum	120.86	38.95	152.29	137.87	46.81	68.61	109.31
	Maximum	12875.92	12123.592	12222.55	9938.28	26732.90	17846.67	9375.92
Alpha-1-Antitrypsin (mg/g)	Median	.422	.405	.400	.398	.366	.407	.475
	Minimum	.040	.048	.070	.035	.020	.055	.084
	Maximum	1.629	2.028	2.773	1.970	2.599	2.590	2.008

%Lactulose, %mannitol and lactulose:mannitol ratio Z-scores assigned average over 3 and 6 months values to age cumulative interval 0-6 months. Myeloperoxidase, neopterin and alpha-1-antitrypsin assigned average values over the cumulative interval 0-6 months.

Myeloperoxidase pairwise comparisons by independent-samples Kruskal-Wallis Test: * EAEC_P3 vs non-pathogens ($p< 0.05$); ** EAEC_P2 vs non-pathogens ($p<0.05$).

Neopterin pairwise comparisons by independent-samples Kruskal-Wallis Test: * EAEC_P3 vs all other groups except ≥3 pathogens without EAEC ($p< 0.05$); ** EAEC_P2 vs non-pathogens and 1 or 2 pathogens without EAEC ($p<0.05$); § ≥3 pathogens without EAEC vs non-pathogens and 1 or 2 pathogens without EAEC ($p<0.05$).

Table 4 - Nutritional impact of enteroaggregative *Escherichia coli* carriage or co-infections with enteric pathogens.

Cumulative nutritional parameters		Groups of children						
		Non pathogens	EAEC_P0*	EAEC_P1	EAEC_P2	EAEC_P3	1 or 2 Pathogens without EAEC	≥3 Pathogens without EAEC
Delta_WLZ	Median	.660	.815	.785	.360	.250*	.700	.940
	Minimum	-4.42	-2.94	-4.70	-3.16	-6.29	-3.29	-2.98
	Maximum	4.37	5.37	5.30	7.15	5.54	4.42	4.42
Delta_WAZ	Median	.320	.310	.280	-.035	-.045*	.350	.480
	Minimum	-2.90	-3.14	-3.11	-2.20	-4.08	-2.81	-3.59
	Maximum	3.62	3.45	6.08	4.83	5.62	5.09	4.14
Delta_LAZ	Median	-.220	-.100	-.080	-.105	-.160	-.015	-.190
	Minimum	-2.74	-2.75	-2.88	-3.23	-3.04	-2.42	-2.11
	Maximum	4.32	5.29	3.18	3.14	3.50	4.76	2.93

Cumulative z-scores (0-6 months follow-up) for weight-for-height (WHZ), weight-for-age (WAZ) and length-for-age (LAZ).

WLZ pairwise comparisons by independent-samples Kruskal-Wallis Test: * EAEC_P3 vs all other groups except EAEC_P2 ($p < 0.05$).

WAZ pairwise comparisons by independent-samples Kruskal-Wallis Test: * EAEC_P3 vs all other groups except EAEC_P2 and non-pathogens which was borderline $p=0.056$ ($p < 0.05$).