Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED)

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Summary

Background Most studies of the causes of diarrhoea in low-income and middle-income countries have looked at severe disease in people presenting for care, and there are few estimates of pathogen-specific diarrhoea burdens in the community.

Methods We undertook a birth cohort study with not only intensive community surveillance for diarrhoea but also routine collection of non-diarrhoeal stools from eight sites in South America, Africa, and Asia. We enrolled children within 17 days of birth, and diarrhoeal episodes (defined as maternal report of three or more loose stools in 24 h, or one loose stool with visible blood) were identified through twice-weekly home visits by fieldworkers over a follow-up period of 24 months. Non-diarrhoeal stool specimens were also collected for surveillance for months 1–12, 15, 18, 21, and 24. Stools were analysed for a broad range of enteropathogens using culture, enzyme immunoassay, and PCR. We used the adjusted attributable fraction (AF) to estimate pathogen-specific burdens of diarrhoea.

Findings Between November 26, 2009, and February 25, 2014, we tested 7318 diarrhoeal and 24 310 non-diarrhoeal stools collected from 2145 children aged 0–24 months. Pathogen detection was common in non-diarrhoeal stools but was higher with diarrhoea. Norovirus GII (AF 5·2%, 95% CI 3·0–7·1), rotavirus (4·8%, 4·5–5·0), *Campylobacter* spp (3·5%, 0·4–6·3), astrovirus (2·7%, 2·2–3·1), and *Cryptosporidium* spp (2·0%, 1·3–2·6) exhibited the highest attributable burdens of diarrhoea in the first year of life. The major pathogens associated with diarrhoea in the second year of life were *Campylobacter* spp (7·9%, 3·1–12·1), norovirus GII (5·4%, 2·1–7·8), rotavirus (4·9%, 4·4–5·2), astrovirus (4·2%, 3·5–4·7), and *Shigella* spp (4·0%, 3·6–4·3). Rotavirus had the highest AF for sites without rotavirus vaccination and the fifth highest AF for sites with the vaccination. There was substantial variation in pathogens according to geography, diarrhoea severity, and season. Bloody diarrhoea was primarily associated with *Campylobacter* spp and *Shigella* spp, fever and vomiting with rotavirus, and vomiting with norovirus GII.

Interpretation There was substantial heterogeneity in pathogen-specific burdens of diarrhoea, with important determinants including age, geography, season, rotavirus vaccine usage, and symptoms. These findings suggest that although single-pathogen strategies have an important role in the reduction of the burden of severe diarrhoeal disease, the effect of such interventions on total diarrhoeal incidence at the community level might be limited.

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Introduction

Infectious diarrhoea is the second most common cause of death in children under 5 years old in developing countries.[1] Studies of the causes of diarrhoea in these settings have usually focused on children who present to health centres and, therefore, best describe pathogens associated with severe diarrhoea.[2,3] However this approach captures only a small subset of diarrhoeal episodes which might show a different hierarchy of pathogens from that associated with mild or moderate episodes of diarrhoea.

Non-severe episodes in the community are of substantial public health importance because of their high prevalence and association with poor growth, impaired cognitive development, environmental enteropathy, and even mortality.[3–8] Estimates of the pathogen-specific burdens of diarrhoea at the community level are, therefore, needed to prioritise interventions. Further, surveillance in the community allows for unbiased estimates of the associations between pathogens and distinct clinical syndromes.

The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Project (MAL-ED) is a multisite birth cohort study at eight sites in South America, sub-Saharan Africa, and Asia.[9] We aimed to estimate pathogen-specific burdens of diarrhoea in children aged 0–24 months at these MAL-ED study sites.

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Research in context

Evidence before this study

We searched PubMed for articles published in any language since 1990 using the terms "diarrhea/diarrhoea" and "etiology/aetiology" and "pediatric/paediatric OR infant*" and "case-control study OR cohort study." We identified 482 publications, including 11 aetiologic studies of diarrhoea which included testing for a broad range of enteropathogens. Of those, eight studied children with more severe diarrhoea presenting to health-care settings. The three remaining studies of community diarrhoea involved a single site.

Added value of this study

Our study provides multisite data on the causes of diarrhoea with longitudinal surveillance and interrogation of a broad range of pathogens, allowing unbiased estimates of pathogen-specific burdens of diarrhoea in the community as well as estimates for specific diarrhoeal syndromes. It documents the broad range of pathogens associated with diarrhoea of any severity, the heterogeneity of the main causes of diarrhoea in low-income and middle-income countries, and the diversity of pathogens associated with seasonal peaks. It also documents the effect of rotavirus vaccine.

Implications of all available evidence

These data suggest that the causes of community diarrhoea are diverse, and single pathogen interventions might not have a substantial impact on total diarrhoeal incidence across multiple populations.

Methods

Study design and participants

A detailed description of the MAL-ED study design is available elsewhere.[9] We enrolled children from the community within 17 days of birth at eight study locations: Dhaka, Bangladesh; Fortaleza, Brazil; Vellore, India; Bhaktapur, Nepal; Loreto, Peru; Naushero Feroze, Pakistan; Venda, South Africa; and Haydom, Tanzania.[10–17]

Inclusion criteria included: a mother aged 16 years or older; intention for the family to stay in the study area for at least 6 months from enrolment; that the child was from a singleton pregnancy and had no other siblings enroled in the study; and birthweight or enrolment weight greater than 1500g. We excluded children with diagnosed congenital disease or severe neontal disease in the newborn.

Enrolment took place between November, 2009, and February, 2012. We aimed to enrol at least 200 children at every site, and we staggered enrolment to capture approximately equal number of births in each calendar month. Follow-up was for 24 months. Length, weight, and head circumference were measured every month, as described previously.[18]

All sites received ethics approval from their respective governmental, local institutional, and collaborating institutional ethics review boards. Written informed consent was obtained from the parent or guardian of every child.

	1 point	2 points	3 points
Duration	2–4 days	5–7 days	≥8 days
Maximum number of loose stools in 24 h	<5 loose stools	5–7 loose stools	>7 loose stools
Days of vomiting	1 day	2 days	>2 days
Presence of dehydration	..	Some dehydration	Severe dehydration
Fever	Maternal report of fever	..	Temperature >37·5°C confirmed by field worker

Elements derived from the Vesikari score[20]

Table 1: **Scoring system for diarrhoea severity score**

Sample and data collection

Non-diarrhoeal stool specimens were collected for surveillance for months 1–12, 15, 18, 21, and 24. Diarrhoeal episodes were collected from age 0–23 months and were identified at home visits made by fieldworkers twice a week. They were defined as maternal report of three or more loose stools in 24 h, or one loose stool with visible blood.[19] Discrete episodes had at least 2 intervening days without diarrhoea. Diarrhoeal stool specimens had to be collected within 48 h of an episode. When a stool sample was collected between two episodes of diarrhoea that met criteria for collection, we assigned the sample to the episode closest to the time of collection.

A diarrhoea severity score was calculated for every episode using elements derived from the Vesikari score (table 1).[20]

Dehydration was defined as irritability that was difficult to console, increased thirst, loss of skin turgor, sunken eyes, or lethargy.[21] Dysentery was defined as diarrhoea in which visible blood was reported by the child's mother. Diarrhoea associated with fever was defined as diarrhoea with fieldworker-confirmed temperature greater than 37·5°C, and vomiting-associated diarrhoea required vomiting at any point during the episode of diarrhoea.

Diarrhoeal episodes of fewer than 7 days' duration were classified as acute, 7–14 days as prolonged, and more than 14 days as persistent. Stools collected within 1 day of administration of a lactulose-mannitol test were excluded from analysis.[22] Data on rotavirus vaccine administration and antibiotic use were recorded and children were referred to medical care for severe symptoms.[23,24]

Stool testing

All stools were analysed in accordance with a standardised microbiology protocol, which was implemented at all

	Children enrolled	Diarrhoea episodes reported	Diarrhoea episode stools collected	Diarrhoeal stools completely tested	Surveillance stools collected	Surveillance stools completely tested	Completely tested diarrhoeal stool samples for specific syndromes							
							Acute (<7 days)	Prolonged (≥7 days)	Mild (score 1–3)	Moderate (score 4–6)	Severe (score >6)	Blood in stool	Associated fever	Associated vomiting
Dhaka, Bangladesh	265	1684	1591	1526 (95·9%)	2937	2910 (99·1%)	1350 (88·5%)	176 (11·5%)	753 (49·3%)	574 (37·6%)	199 (13·0%)	64 (4·2%)	48 (3·2%)	477 (31·3%)
Vellore, India	251	982	749	698 (93·2%)	3215	3181 (98·9%)	611 (87·5%)	87 (12·5%)	406 (58·2%)	218 (31·2%)	74 (10·6%)	49 (7·0%)	13 (1·9%)	164 (23·5%)
Bhaktapur, Nepal	240	1083	976	925 (94·8%)	3105	3071 (98·9%)	684 (74·0%)	241 (26·1%)	266 (28·8%)	525 (56·8%)	134 (14·5%)	43 (4·7%)	58 (6·3%)	179 (19·4%)
Naushero Feroze, Pakistan	277	3255	2272	1836 (80·8%)	2820	2777 (98·5%)	1182 (64·4%)	654 (35·6%)	498 (27·1%)	770 (41·9%)	568 (30·9%)	60 (3·3%)	91 (5·0%)	641 (34·9%)
Venda, South Africa	314	324	200	157 (78·5%)	3720	3617 (97·2%)	149 (94·9%)	8 (5·1%)	122 (77·7%)	32 (20·4%)	3 (1·9%)	4 (2·6%)	4 (2·6%)	28 (17·8%)
Haydom, Tanzania	262	625	206	171 (83·0%)	3295	3252 (98·7%)	158 (92·4%)	13 (7·6%)	95 (55·6%)	63 (36·8%)	13 (7·6%)	27 (15·8%)	0	63 (36·8%)
Fortaleza, Brazil	233	188	129	117 (90·7%)	2519	2425 (96·3%)	99 (84·6%)	18 (15·4%)	73 (62·4%)	34 (29·1%)	10 (8·6%)	2 (1·7%)	12 (10·3%)	34 (29·1%)
Loreto, Peru	303	2131	2047	1888 (92·2%)	3185	3077 (96·6%)	1584 (83·9%)	304 (16·1%)	1038 (55·0%)	650 (34·4%)	200 (10·6%)	108 (5·7%)	120 (6·4%)	347 (18·4%)
Total	2145	10272	8170	7318 (89·6%)	24796	24310 (98·0%)	5817 (79·5%)	1501 (20·5%)	3251 (44·4%)	2866 (39·1%)	1201 (16·4%)	357 (4·9%)	346 (4·7%)	1933 (26·4%)

Table 2: **MAL-ED cohort descriptive statistics and completeness of surveillance and testing**

study sites and has been described in detail previously.[25] We used conventional stool culture to identify bacterial pathogens with the exception of *Campylobacter* spp.

Testing for diarrhoeagenic *Escherichia coli* was done by pooling five lactose-fermenting colonies for multiplex PCR to detect the toxin-encoding genes *stx1*, *stx2*, *eae*, *bfpA*, *ipaH*, *aatA*, and *aaiC*, as well as those encoding heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST).

Enzyme immunoassay was used for detection of *Campylobacter* spp, rotavirus, adenovirus, and astrovirus (ProSpecT, Remel, Lenexa, KS, USA) and *Entamoeba histolytica*, *Giardia* spp, and *Cryptosporidium* spp (TechLab, Blacksburg, VA, USA). Rotavirus detections were considered negative if obtained within 28 days of rotavirus vaccine administration (n=18).

We used PCR to test all diarrhoeal stool samples for norovirus. We also aimed to test all non-diarrhoeal stool samples from a randomly selected 10% subset of participants at each site.

If an additional specimen was available, we did use microscopy for identification of protozoa and helminths; however, microscopy was not required for complete testing, and microscopy results were not included for the analysis of infections for the three protozoal pathogens tested by enzyme immunoassay. If testing was incomplete, recollection was allowed within 48 h.

Statistical analysis

Because pathogens were frequently detected in diarrhoeal and non-diarrhoeal stools, we used the adjusted attributable fraction (AF) to estimate pathogen-specific

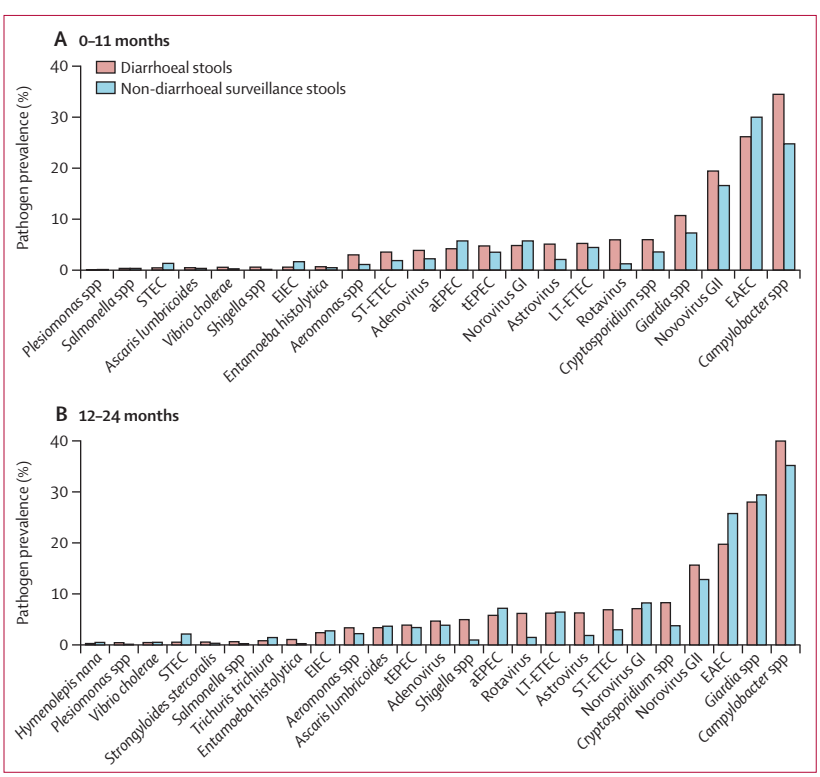

Figure 1: **Pathogens detected in diarrhoeal and non-diarrhoeal stools, 0–11 months and 12–24 months**
EAEC=enteroaggregative *Escherichia coli*; EIEC=enteroinvasive *E coli*; aEPEC=atypical enteropathogenic *E coli*; tEPEC=typical enteropathogenic *E coli*; LT-ETEC=LT-producing enterotoxigenic *E coli*; ST-ETEC=ST-producing enterotoxigenic *E coli*; STEC=Shiga-toxin-producing *E coli*. Pathogens present in less than 0.1% of stool samples are not shown.

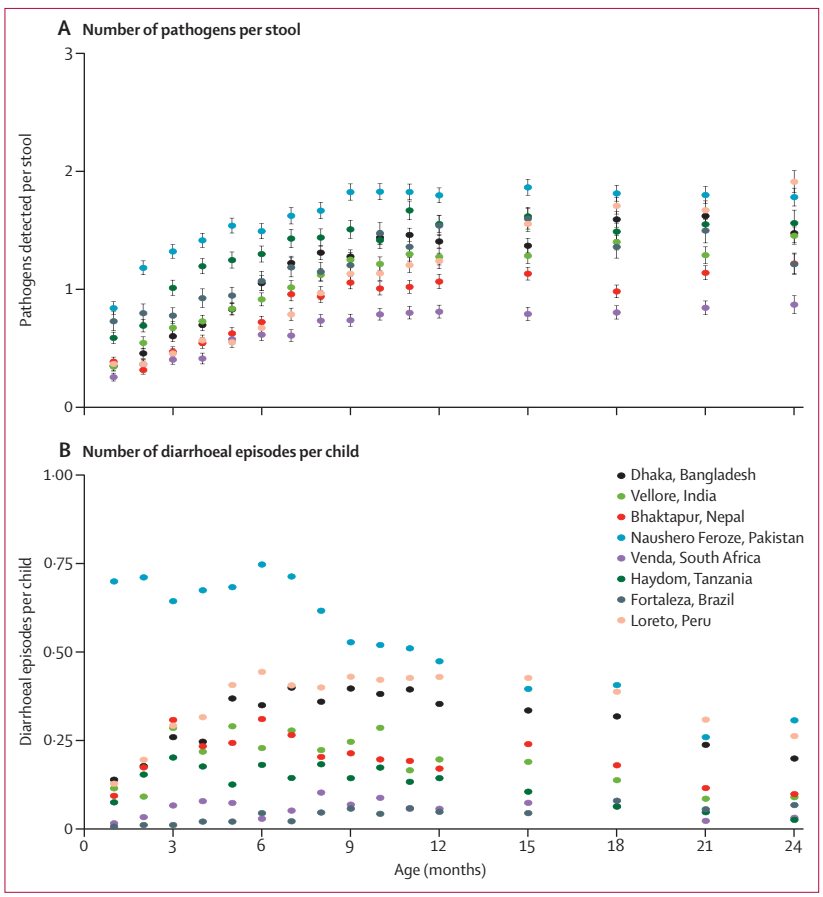

Figure 2: **Pathogen detection and diarrhoeal episodes per child, 0–24 months**
Dots show mean values with standard error bars.

burdens of diarrhoea, a measurement that incorporates the prevalence of detection in diarrhoeal stools and the strength of association with diarrhoea.

To analyse the strength of association between diarrhoea and detection of individual pathogens, we used generalised estimating equations (GEEs) to fit a binary logistic regression model for each site and age group to account for non-independence of stool testing within each participant. All models were adjusted for age (in days), sex, and site. We included all detected pathogens from diarrhoeal stools for each age and site, and we assumed an independent working correlation matrix. We then calculated AFs using the point estimate of the odds ratios derived from the multivariate GEEs[26,27] with 95% CIs estimated using the Delta method.[28]

We determined the pathogen-specific attributable incidence for each calendar month by first calculating the AF using the prevalence of each pathogen in diarrhoea for each calendar month and then multiplying by the number of episodes of diarrhoea during that month. To mitigate the detection of convalescent excretion of pathogens, we excluded from analysis non-diarrhoeal stools collected more than 48 h but fewer than 7 days before or after a diarrhoeal episode. The effect of prolonged excretion of enteric pathogens on AF estimates was evaluated by further restricting non-diarrhoeal specimens to those collected at least 28 days before and after any diarrhoeal episode. Pathogen-specific AFs were calculated for the subset of diarrhoeal episodes that met study definitions of acute, prolonged, persistent, mild, moderate, severe, or dysenteric diarrhoea, or diarrhoea associated with fever or with vomiting.

	Dhaka, Bangladesh	Vellore, India	Bhaktapur, Nepal	Naushero Feroze, Pakistan	Venda, South Africa*	Haydom, Tanzania	Fortaleza, Brazil*	Loreto, Peru*	Overall
Age 0–11 months									
Diarrhoeal stools	819	419	524	1230	84	145	38	1021	4280
Non–diarrhoeal stools	2194	2252	2264	1902	2665	2391	1747	2354	17769
Norovirus GII	..	..	8·4% (5·7–9·7)	..	..	8·2% (0·5–12·9)	..	5·1% (0·2–9·1)	5·2% (3·0–7·1)
Rotavirus	9·6% (8·8–10·1)	6·0% (5·5–6·3)	6·6% (5·9–6·9)	3·2% (2·6–3·5)	..	9·5% (7·6–10·5)	..	1·0% (0·0–1·6)	4·8% (4·5–5·0)
Campylobacter spp	..	..	..	..	16·9% (9·0–21·6)	..	30·9% (22·8–34·3)	5·6% (0·7–9·5)	3·5% (0·4–6·3)
Astrovirus	2·0% (0·3–3·2)	4·2% (3·2–4·9)	..	2·2% (0·9–3·1)	..	..	..	3·6% (2·7–4·3)	2·7% (2·2–3·1)
Cryptosporidium spp	..	..	..	3·6% (1·9–4·8)	..	6·3% (1·2–9·1)	5·5% (0·0–7·2)	2·6% (0·6–4·1)	2·0% (1·3–2·6)
ST–ETEC	4·7% (3·3–5·8)	1·7% (0·6–2·3)	2·0% (1·0–2·5)	1·2% (0·1–1·8)	3·3% (0·9–4·2)	..	..	..	1·9% (1·5–2·2)
Adenovirus	..	2·7% (0·9–3·7)	2·3% (0·7–3·2)	1·1% (0·0–1·9)	..	..	..	1·5% (0·2–2·3)	1·6% (1·0–2·0)
tEPEC	2·2% (0·0–4·1)	..	..	..	..	..	..	..	1·3% (0·7–1·9)
LT–ETEC	2·0% (0·2–3·3)	..	..	..	..	..	16·9% (11·1–19·3)	..	1·3% (0·6–1·9)

(Table 3 continues on next page)

	Dhaka Bangladesh	Vellore, India	Bhaktapur, Nepal	Naushero Feroze, Pakistan	Venda South Africa*	Haydom, Tanzania	Fortaleza, Brazil*	Loreto, Peru*	Overall
(Continued from previous page)									
Shigella spp	..	..	0·7% (0·3–0·7)	0·9% (0·6–1·1)	..	..	..	..	0·4% (0·2–0·5)
Age 12–24 months									
Diarrhoeal stools	707	279	401	606	73	26	79	867	3038
Non–diarrhoeal stools	716	929	807	875	952	861	678	723	6541
Campylobacter spp	..	..	8·8% (2·0–13·8)	..	..	..	..	9·9% (3·0–15·5)	7·9% (3·1–12·1)
Norovirus GII	..	..	11·2% (6·4–11·9)	..	19·2% (2·2–26·3)	..	..	11·7% (6·0–15·2)	5·4% (2·1–7·8)
Rotavirus	6·0% (4·8–6·6)	4·8% (4·0–5·2)	8·7% (8·7–8·7)	2·2% (0·7–2·9)	..	14·3% (11·5–15·1)	4·3% (1·7–4·9)	2·9% (0·8–4·2)	4·9% (4·4–5·2)
Astrovirus	2·6% (0·7–3·7)	3·1% (1·7–3·7)	4·6% (3·2–5·3)	..	..	9·7% (1·8–11·2)	4·7% (3·2–5·0)	7·4% (5·5–8·6)	4·2% (3·5–4·7)
Shigella spp	1·5% (0·3–2·0)	9·4% (8·7–9·8)	6·8% (5·8–7·4)	5·1% (3·8–5·9)	..	..	3·7% (2·1–3·8)	2·1% (0·8–2·7)	4·0% (3·6–4·3)
ST–ETEC	8·0% (5·6–9·7)	5·4% (3·6–6·3)	4·6% (2·2–5·9)	..	..	9·1% (2·7–10·9)	..	2·0% (0·5–2·7)	3·9% (3·1–4·5)
Cryptosporidium spp	2·5% (0·0–4·0)	6·9% (5·3–7·7)	3·2% (1·4–4·1)	5·5% (3·5–6·8)	..	13·0% (6·9–14·7)	..	..	3·8% (2·8–4·7)
LT–ETEC	2·4% (0·1–3·8)	..	..	..	..	16·1% (0·0–22·8)	..	..	1·2% (0·0–2·1)
Adenovirus	..	3·6% (0·9–5·0)	3·9% (2·1–4·8)	..	..	..	3·8% (1·1–4·7)	..	0·9% (0·0–1·8)
EIEC	..	..	1·2% (0·0–1·6)	..	..	..	..	..	0·8% (0·1–1·2)
Entamoeba histolytica	..	0·7% (0·7–0·7)	..	0·8% (0·2–1·1)	..	..	..	..	0·7% (0·3–0·9)
Salmonella	..	0·7% (0·7–0·7)	0·5% (0·5–0·5)	..	..	..	..	0·5% (0·5–0·5)	0·3% (0·0–0·5)
Norovirus GI	..	..	1·0% (1·0–1·0)	..	..	..	..	..	..
Aeromonas	..	..	..	..	..	..	..	1·0% (0·1–1·2)	..
Plesiomonas	..	0·7% (0·7–0·7)	..	..	..	..	..	..	..
STEC	..	..	..	..	..	..	..	0·2% (0·2–0·2)	..

EIEC=enteroinvasive *Escherichia coli*; tEPEC=typical enteropathogenic *E coli*; LT-ETEC=LT-producing enterotoxigenic *E coli*; ST-ETEC=ST-producing enterotoxigenic *E coli*; STEC=Shiga-toxin producing *E coli*. Data are n or attributable fractions (95% CI). For cells with ··, the pathogen was either not detected or was not statistically significantly associated with diarrhoea (appendix). *Monovalent rotavirus vaccine was introduced to the national immunisation programme at these sites before the study began.

***Table 3*: Adjusted attributable fraction of diarrhoea for individual pathogens in the first and second year of life**

To analyse the association between pathogen detection and diarrhoea severity, GEEs were used to fit an ordinal regression model which was specified identically to the logistic regression models used for the analysis of diarrhoea association. For all analyses, we constructed models both with and without norovirus because of the differential testing of non-diarrhoeal specimens for this pathogen. The results we report for pathogens other than norovirus, as well as for all analyses involving aggregated pathogen testing, were derived from models that excluded norovirus. We used R version 3.0.3 (Foundation for Statistical Computing, Vienna, Austria) for all statistical analyses, with the geepack package within this program used for GEE analysis.[29]

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Nov 3, 2009, and 29 February, 2012, we enrolled 2145 children (range 233–314 per site). The size of the

See Online for appendix

cohort at each site and completeness of stool testing is shown in table 2. We recorded 2 years of follow-up data for 1740 participants (81·1%).

Two fieldworker visits per week were sufficient to collect most diarrhoeal stools within 48 h (79·5% overall; site range 33·0–96·1%). Collection rates were higher for longer episodes (75·5% for acute episodes and 99·3% for prolonged or persistent episodes).

A broad range of pathogens was detected, with 22 pathogens in the first year of life and 25 in the second year of life (we have not included pathogens in analysis if they were present in only very few samples—ie, less than 0·1% of all stools). For certain pathogens, detection in non-diarrhoeal stools approached, and in some cases exceeded, that noted for diarrhoeal stools (figure 1).

Enteropathogen infection began soon after birth and was common at all sites; however, the intensity varied between sites, ranging from an average of about 0·5 pathogens detected per stool by the end of the first year of life (South Africa) to almost two pathogens per stool (Pakistan; figure 2). Both the incidence of diarrhoea and the number of pathogens detected per stool increased markedly during the first year of life. At least one pathogen was detected in 76·9% (n= 15767) of diarrhoeal stools and 64·9% (15767) of non-diarrhoeal stools, and two or more pathogens were identified in 41·0% (2999) and 29·0% (7046) of stools, respectively. The number of pathogens detected was higher in diarrhoeal stools than non-diarrhoeal stools at most time points (appendix).

The presence of pathogens was associated with diarrhoea, in that each additional pathogen increased the odds of diarrhoea (odds ratio (OR) 1·20 per pathogen detection, p<0·0001]). Antibiotics were administered for 4696 (46%) diarrhoeal episodes captured by surveillance with a range between sites of 20 (11%, Brazil) to 1922 (59%, Pakistan).

Overall, 19·1%, (95% CI 16·2–21·8) and 33·1% (29·0–36·7) of diarrhoeal episodes in the first and second year of life, respectively, could be attributed to pathogens. Attributable fractions did not change appreciably when the more restrictive definition of non-diarrhoeal specimens was applied, suggesting that estimates were not biased by convalescent excretion (appendix), nor did they change after controlling for child nutritional status (height-for-age *Z* score).

Across all sites and episodes, the highest AFs were seen for norovirus GII, rotavirus, *Campylobacter* spp, astrovirus, and *Cryptosporidium* spp in the first year of life and *Campylobacter* spp, norovirus GII, rotavirus, astrovirus, and *Shigella* spp in the second year of life (table 3 and appendix).

There was substantial heterogeneity between sites in the individual pathogen most often associated with diarrhoea, with the highest burden of diarrhoea attributed to four unique pathogens in the first year of life (*Campylobacter* spp, *Cryptosporidium* spp, norovirus GII,

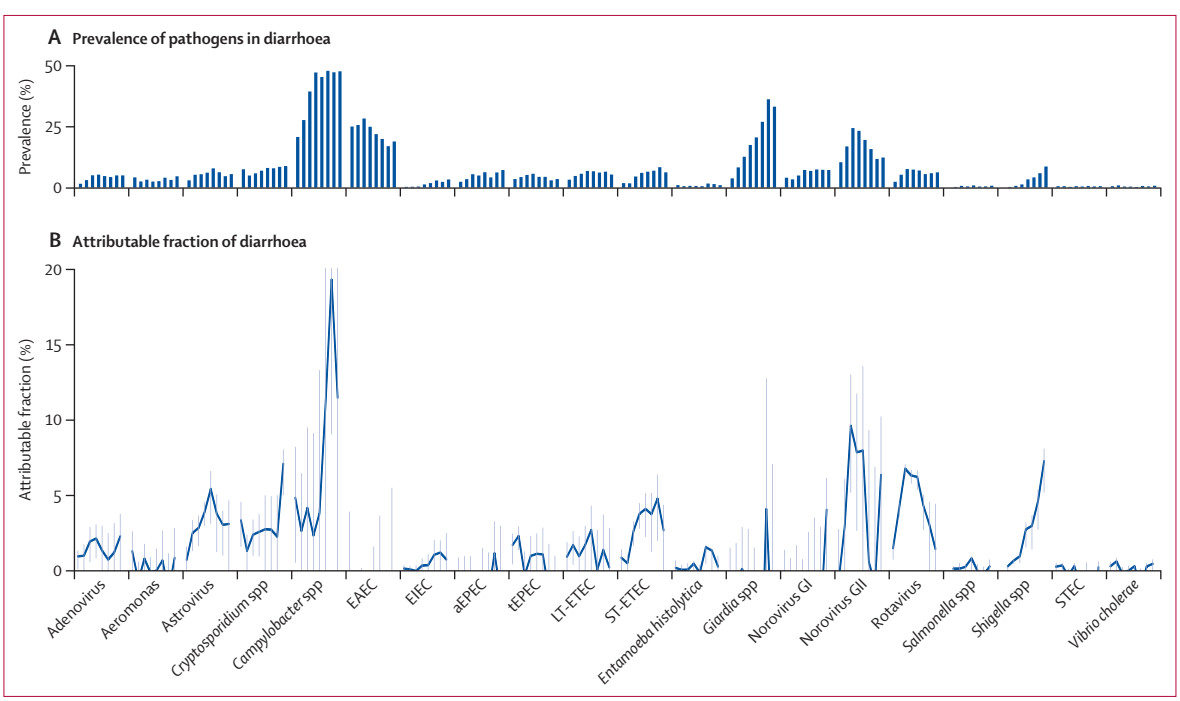

Figure 3: **Prevalence and adjusted attributable fraction of diarrhoea for 3-month intervals, age 0–24 months**
EAEC=enteroaggregative *Escherichia coli*; EIEC=enteroinvasive *E coli*; aEPEC=atypical enteropathogenic *E coli*; tEPEC=typical enteropathogenic *E coli*; LT-ETEC=LT-producing enterotoxigenic *E coli*; ST-ETEC=ST-producing enterotoxigenic *E coli*; STEC=Shiga-toxin producing *E coli*. Data are attributable fractions (95% CI). For each organism, the first data point represents age 0–2 months, the second represents age 3-5 months, then 6–8 months, 9–11 months, 12–14 months, 15-17 months, 18–20 months, and 21–24 months.

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and rotavirus) and six across the eight sites in the second year of life (astrovirus, *Cryptosporidium* spp, LT-producing enterotoxigenic *E coli*, norovirus GII, *Shigella* spp, and ST-producing enterotoxigenic *E coli*; table 3) . The monovalent rotavirus vaccine was introduced in three participating countries (South Africa, Brazil, and Peru) before the study began, with 89·4% of enrolled children receiving at least one dose at those sites. The effect of rotavirus vaccine was evident, in that rotavirus had the highest overall AF at sites without rotavirus vaccination (AF 5·8%, 95% CI 5·6–6·0) and the fifth highest overall AF at sites with rotavirus vaccination (1·9%, 1·0–2·6).

Three frequently detected pathogens, namely enteroaggregative *E coli*, *Giardia* spp, and atypical enteropathogenic *E coli*, were not statistically significantly associated with diarrhoea for any age group, site, or diarrhoeal syndrome. Age-related patterns were seen for several pathogens: astrovirus, norovirus GII, and rotavirus diarrhoea burdens peaked during age 6–12 months, whereas *Cryptosporidium* spp, *Shigella* spp, *Campylobacter* spp, and ST-producing enterotoxigenic *E coli* continued to increase through the second year of life (figure 3). First infections were more strongly associated with diarrhoea than were subsequent infections for most pathogens; however, this did not alter AF estimates (data not shown). Helmintic infections were not associated with diarrhoea for any age group, site, or diarrhoeal syndrome.

We next examined whether clinical characteristics or seasonality could aid prediction of the cause of diarrhoea. Total attribution to pathogens for episodes associated with dysentery, dehydration, or admission to hospital was 33·4% (95% CI 27·1–38·6) and 29·1% (26·6–31·0%) in the first and second year of life, respectively, and pathogens most often associated with these events were rotavirus, *Campylobacter* spp, and norovirus GII in the first year and *Shigella* spp, rotavirus, and ST-producing enterotoxigenic *E coli* in the second year of life (appendix). *Campylobacter, Shigella* spp, and enteroinvasive *E coli* were associated with the highest burden of dysentery (table 4). Pathogens associated with fever included rotavirus and *Shigella* spp. Rotavirus and norovirus GII were the pathogens most often associated with vomiting.

Use of the diarrhoea severity score that incorporated vomiting, fever, frequency, and dehydration showed that the following were associated with a higher severity score: rotavirus (OR 2·30 per one unit increase in severity score, 95% CI 1·91–2·77; p<0·0001), *Shigella* spp (1·48, 1·13–1·93; p=0·0043), adenovirus (1·45, 1·19–1·78;

	Acute (<7 days)	Prolonged (≥7 days)	Mild (score 1–3)	Moderate (score 4–6)	Severe (score >6)	Blood in stool	Associated fever	Associated vomiting	Overall
Age 0–11 months									
Diarrhoeal stools (% of diarrhoea)	3249 (75·9%)	1031 (24·1%)	1696 (39·6%)	1762 (41·2%)	820 (19·2%)	198 (4·6%)	204 (4·8%)	1235 (28·9%)	4280
Norovirus GII	5·5% (3·1–7·5)	4·4% (0·9–7·2)	5·2% (2·5–7·6)	4·7% (2·0–7·0)	5·5% (1·8–8·5)	..	..	7·5% (4·5–10·0)	5·2% (3·0–7·1)
Rotavirus	5·6% (5·3–5·8)	2·2% (1·7–2·6)	2·0% (1·5–2·3)	5·2% (4·9–5·5)	9·8% (9·5–10·1)	..	7·2% (6·3–7·7)	11·1% (10·8–11·4)	4·8% (4·5–5·0)
Campylobacter spp	4·4% (1·1–7·3)	..	8·1% (4·3–11·4)	..	..	23·7% (14·2–30·3)	..	..	3·5% (0·4–6·3)
Astrovirus	2·9% (2·4–3·4)	1·8% (0·8–2·5)	2·7% (2·0–3·2)	2·3% (1·6–2·9)	3·4% (2·4–4·1)	..	..	3·9% (3·1–4·5)	2·7% (2·2–3·1)
Cryptosporidium spp	1·7% (0·9–2·4)	3·0% (1·8–4·0)	1·2% (0·0–2·0)	2·3% (1·3–3·1)	3·1% (1·5–4·2)	..	..	2·4% (1·1–3·4)	2·0% (1·3–2·6)
ST-ETEC	2·4% (1·9–2·7)	..	1·8% (1·2–2·3)	2·2% (1·7–2·6)	1·4% (0·5–2·0)	..	..	1·9% (1·2–2·5)	1·9% (1·5–2·2)
Adenovirus	1·4% (0·8–1·9)	2·1% (1·2–2·7)	1·0% (0·3–1·5)	1·6% (0·9–2·2)	3·2% (2·2–3·9)	..	3·0% (0·9–4·1)	3·1% (2·2–3·7)	1·6% (1·0–2·0)
tEPEC	1·2% (0·4–1·8)	1·6% (0·5–2·5)	1·4% (0·4–2·2)	..	2·2% (0·8–3·2)	..	..	1·5% (0·2–2·5)	1·3% (0·7–1·9)
LT-ETEC	0·9% (0·1–1·6)	2·6% (1·4–3·4)	1·0% (0·0–1·8)	1·1% (0·1–1·9)	2·3% (0·9–3·3)	..	..	1·8% (0·6–2·8)	1·3% (0·6–1·9)
Shigella spp	0·3% (0·1–0·4)	0·6% (0·3–0·7)	0·3% (0·1–0·4)	0·4% (0·2–0·5)	..	3·4% (3·1–3·5)	1·2% (0·5–1·4)	..	0·4% (0·2–0·5)
STEC	..	0·5% (0·0–0·7)	..	..	..	..	..	..	..
EIEC	..	..	..	..	0·8% (0·4–1·0)	1·7% (0·4–2·2)	..	..	..
Salmonella spp	..	..	..	..	0·6% (0·1–0·9)	..	1·5% (0·6–1·8)	..	..
Entamoeba histolytica	..	..	..	..	..	..	1·3% (0·0–1·7)	..	..

(Table 4 continues on next page)

	Acute (<7 days)	Prolonged (≥7 days)	Mild (score 1–3)	Moderate (score 4–6)	Severe (score >6)	Blood in stool	Associated fever	Associated vomiting	Overall
(Continued from previous page)									
Age 12–24 months									
Diarrhoeal stools (% of diarrhoea)	2568 (84·5%)	470 (15·5%)	1553 (51·1%)	1104 (36·3%)	381 (12·5%)	159 (5·2%)	142 (4·7%)	698 (23·0%)	3038
Campylobacter spp	8·9% (4·0–13·2)	..	9·7% (3·9–14·7)	8·3% (1·8–13·9)	..	..	..	..	7·9% (3·1–12·1)
Norovirus GII	5·1% (1·8–7·6)	6·9% (1·4–10·4)	4·5% (0·7–7·3)	6·2% (2·3–9·0)	6·9% (1·3–10·4)	..	..	8·9% (4·9–11·7)	5·4% (2·1–7·8)
Rotavirus	5·2% (4·7–5·6)	2·9% (1·9–3·4)	3·8% (3·2–4·3)	5·1% (4·5–5·5)	7·9% (7·2–8·4)	..	4·9% (3·4–5·7)	10·1% (9·6–10·5)	4·9% (4·4–5·2)
Astrovirus	4·5% (3·8–5·0)	2·3% (0·9–3·2)	4·1% (3·2–4·7)	4·7% (3·9–5·3)	2·8% (1·1–3·7)	..	5·4% (3·2–6·6)	4·5% (3·5–5·2)	4·2% (3·5–4·7)
Shigella spp	3·4% (3·0–3·7)	7·0% (6·4–7·4)	2·7% (2·2–3·0)	5·1% (4·6–5·5)	5·7% (5·0–6·1)	17·2% (16·5–17·6)	6·9% (6·0–7·3)	3·1% (2·4–3·4)	4·0% (3·6–4·3)
ST-ETEC	3·6% (2·8–4·3)	5·5% (4·1–6·4)	3·4% (2·5–4·2)	3·9% (2·8–4·8)	5·8% (4·4–6·8)	..	3·6% (0·6–5·0)	5·5% (4·4–6·3)	3·9% (3·1–4·5)
Cryptosporidium spp	3·4% (2·2–4·3)	6·1% (4·1–7·4)	3·0% (1·6–4·2)	4·5% (3·1–5·6)	3·2% (0·5–4·9)	..	..	3·8% (1·8–5·1)	3·8% (2·8–4·7)
LT-ETEC	1·3% (0·1–2·3)	..	..	1·5% (0·0–2·8)	..	..	5·0% (1·2–7·1)	2·2% (0·3–3·4)	1·2% (0·0–2·1)
Adenovirus	1·0% (0·2 –1·9)	..	0·8% (0·1–1·3)	1·9% (0·4–3·0)	..	..	..	1·9% (0·0–3·1)	0·9% (0·0–1·8)
EIEC	0·8% (0·1–1·3)	..	0·9% (0·5–1·1)	1·2% (0·2–1·8)	..	5·0% (3·2–5·8)	..	..	0·8% (0·1–1·2)
E histolytica	0·7% (0·3–0·9)	..	1·1% (0·7–1·3)	..	..	..	..	..	0·7% (0·3–0·9)
Salmonella	0·4% (0·1–0·5)	..	0·4% (0·1–0·5)	..	..	..	1·8% (1·1–2·0)	..	0·3% (0·0–0·5)
Aeromonas spp	..	..	..	..	..	3·3% (1·0–4·3)	..	..	..
Plesiomonas spp	..	..	..	..	..	1·2% (0·0–1·6)	..	..	..

EIEC=enteroinvasive *Escherichia coli*; tEPEC=typical enteropathogenic *E coli*; LT-ETEC=LT-producing enterotoxigenic *E coli*; ST-ETEC=ST-producing enterotoxigenic *E coli*; STEC=Shiga-toxin producing *E coli*. Data are n or attributable fractions (95% CI). The subset of pathogens assayed that were significant in at least one syndrome or age group are shown in descending order of average attributable fraction for study-defined diarrhoea. For cells with a dash, the pathogen was either not detected or was not statistically significantly associated with diarrhoea.

Table 4: **Adjusted attributable fraction of diarrhoea associated with specific diarrhoeal syndromes in the first and second year of life for individual pathogens**

p=0·0003), and *Cryptosporidium* spp (1·26, 1·07–1·49; p=0·0065). *Campylobacter* spp were associated with a lower score (0·85, 0·77–0·94; p=0·0011) .

Persistent diarrhoea represented 4·9% and 1·8% of episodes during the first and second year of life, respectively, and was associated with LT-producing enterotoxigenic *E coli*, astrovirus, *Cryptosporidium* spp, ST-producing enterotoxigenic *E coli*, and *Shigella* spp in the first year of life and *Shigella* and astrovirus in the second (data not shown).

The association between the attributable incidence of specific pathogens and seasonal diarrhoeal incidence varied between sites (figure 4). For many sites, peak diarrhoeal incidence coincided with the peak attributable incidence for some pathogens—for example *Cryptosporidium* spp, ST-producing enterotoxigenic *E coli*, *Shigella* spp, and astrovirus in India and norovirus GII, ST-producing enterotoxigenic *E coli*, and *Shigella* spp in Nepal. Rotavirus incidence was strongly seasonal, and during peak season it dominated all-cause diarrhoea incidence in India, Nepal, Pakistan, and Tanzania. There was little association between rotavirus incidence and seasonality at the three sites where rotavirus vaccine had been introduced.

Discussion

In this multicountry community-based cohort study, pathogen-specific burdens of diarrhoea varied substantially between sites. Although rotavirus diarrhoea burden was substantially decreased at sites where rotavirus vaccine had been introduced, it occupied the overall highest burden of disease at the five sites that do not have vaccination. Nevertheless, it was associated with the highest burden of diarrhoea at only three sites in the first year of life and at none in the second year. *Cryptosporidium* spp, ST-producing enterotoxigenic *E coli*, and *Shigella* spp were also associated with more severe diarrhoea than were other pathogens and are well known to be important pathogens.[2,3] Additionally, however, a substantial number of diarrhoeal episodes were attributable to *Campylobacter* spp, norovirus GII, and

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astrovirus—pathogens that have rarely been examined in such a large study with modern diagnostic tools,[2] or have not been noted as important in case-control studies.[2,3,30] The number and diversity of pathogens associated with community diarrhoea suggests that single pathogen interventions, apart from rotavirus vaccination, might not have an effect on the incidence of diarrhoeal episodes across populations.

This multisite longitudinal study design allowed us to uncover an unbiased picture of the association between specific pathogens and specific clinical features, including duration, severity, dysentery, febrile illness, and vomiting.

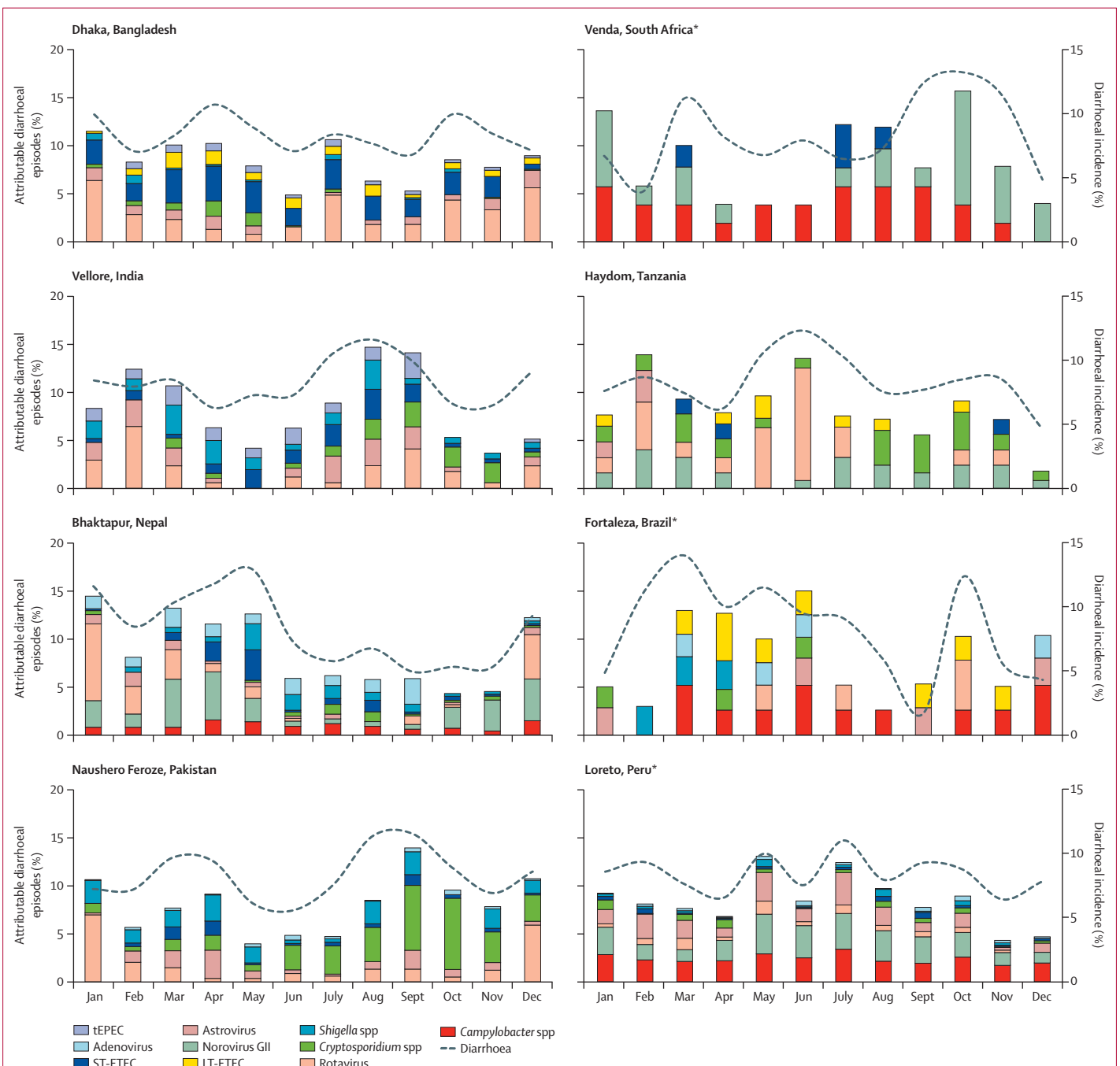

Figure 4: **Association between individual pathogens and seasonal diarrhoeal incidence**
tEPEC=typical enteropathogenic *Escherichia coli*; LT-ETEC=LT-producing enterotoxigenic *E coli*; ST-ETEC=ST-producing enterotoxigenic *E coli*. Primary y-axis shows percent of total attributable incidence of diarrhoea for individual pathogens; secondary y-axis (and dotted line) shows annual diarrhoeal incidence by calendar month. *Monovalent rotavirus vaccine was introduced to the national immunisation programme before the study began.

Dysentery in the first year of life was predominantly associated with *Campylobacter* spp; however, Campylobacter-associated diarrhoea was, otherwise, mild when assessed with a severity score that did not include the presence of blood. By contrast, dysentery associated with *Shigella* spp was often severe and of surprisingly long duration. Rotavirus and norovirus GII were associated with vomiting.

Campylobacter spp were the most frequently detected pathogens and had the highest burden of diarrhoea in Brazil, Peru, and South Africa in the first year of life. Such a high burden of *Campylobacter* spp early in the first year of life, often with dysentery, has been observed in some studies but not others.[2] This pathogen did not show strong seasonal trends. We have previously shown that culture substantially underdetects *Campylobacter*[31] whereas EIA broadly detects *Campylobacter* spp, including species other than *C jejuni* and *C coli*. We expect most of the episodes associated with *Campylobacter* spp to be caused by *C jejuni or C coli*, but culture identification was only done on a subset of stools in our study and further work is needed.

We documented a substantial burden of diarrhoea associated with norovirus GII infection at the sites in Nepal, South Africa, Tanzania, and Peru, as well as in the overall analysis. As in developed countries,[32] norovirus GII appeared to be a significant contributor to overall diarrhoeal incidence at several sites. There has been substantial variation in previous estimates of the global burden of norovirus, in part because detection of norovirus GII is often high in asymptomatic control participants matched for age, community, and season.[30]

Astrovirus is known to be a common cause of sporadic diarrhoea that is less severe than that associated with rotavirus, and astrovirus often exists as a co-infection.[33,34] Our study shows the global importance of astrovirus diarrhoea, with a substantial burden of disease in most sites. Adenovirus had a low overall attributable fraction, but, when present, was associated with diarrhoea classified as "severe" by an adapted Vesikari score. We used a pan-adenovirus ELISA without typing for the major gastrointestinal subtypes 40/41; however, we would not expect the AF for adenovirus to increase significantly given its low prevalence. Helminth infections were rare in this study, except for *Ascaris* in the second year of life, and were not associated with diarrhoea.

This study also documents frequent detection of a wide range of pathogens, including *Campylobacter* spp, enteroaggregative *E coli*, norovirus, *Giardia*, LT-producing enterotoxigenic *E coli*, and typical and atypical enteropathogenic *E coli* in routinely collected non-diarrhoeal stools. Whether the presence of these pathogens is associated with more insidious phenotypes such as poor growth, impaired cognitive development, environmental enteropathy, or impaired mucosal immunity is unclear and further study is warranted in this area.

Our study has some limitations. In light of the variation between sites in diarrhoeal incidence, the study was not powered to identify all associations between pathogens and diarrhoea at individual sites. Furthermore, because short episodes of diarrhoea are more difficult to capture with community-based surveillance than are longer periods of diarrhoea, especially in rural settings, burden estimates might be biased against pathogens associated with a short duration of symptoms. Additionally, we used a modified severity score that only partly recapitulates a score derived from rotavirus studies and may not be generalisable. Therefore, we also looked at the subset of diarrhoea associated with dysentery, dehydration, or hospital admission in addition to looking at specific diarrhoeal syndromes. Finally, the diagnostic approach used a diverse set of detection methods with differing performance characteristics. It is possible, for example, that culture for bacterial pathogens is insensitive and was affected by the frequent use of antibiotics for diarrhoea in these settings, such that the use of culture for detection may have resulted in underestimates of bacterial presence. Molecular testing, in particular quantification of pathogen load and quantitative analysis, could revise estimates of the burden of diarrhoea for these organisms.[35]

The longitudinal nature of this study allowed us to look at causes of diarrhoea in ways that are not possible with other study designs, including use of unbiased estimates of causes of diarrhoea at the community level and evaluation of assumptions about appropriate control specimens.[36] Detection of pathogens in non-diarrhoeal stool samples might represent convalescent excretion of certain pathogens rather than true asymptomatic infection, in which case we may underestimate the burden of diarrhoea associated with these organisms. Malnourished children may be particularly likely to have prolonged excretion of enteropathogens. However, controlling for nutritional status did not appreciably alter AF estimates.

This study documents a diverse range of pathogens associated with community diarrhoea in children in low-income and middle-income countries, which contrasts with the smaller set of pathogens associated with severe diarrhoea. The hierarchy of pathogen-specific diarrhoea varied between sites and high rates of enteropathogens were detected in non-diarrhoeal samples.

Consistent with previous studies,[2,3] a high burden of childhood diarrhoea was attributed to rotavirus, ST-ETEC, *Shigella* spp, and *Cryptosporidium* spp. However, our results suggest that *Campylobacter* spp, norovirus GII, and astrovirus also contribute substantially to the burden of diarrhoea in children.

Contributors

JPM, BJM, MMcGrath, JDC and SR participated in data management and data analysis. SB, LB, JG, RH, AH, MO, AS, SS, DM, IFL, DH, BBR, SQ, FK, PPY, BM, and CA performed and supervised laboratory testing and data collection. PB, EM, TA, AAL, CJM, AZ, ZB, MK, RLG, GK, DL supervised the study. MG and MM organised the project and acquired grant funds. JPM and ERH wrote the report with input from all authors. ERH had final responsibility for the decision to submit for publication. All authors reviewed the draft and approved the decision to submit for publication.

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Declaration of interests
We declare no competing interests.

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References

1 Walker CL, Aryee MJ, Boschi-Pinto C, Black RE. Estimating diarrhea mortality among young children in low and middle income countries. *PLoS One* 2012; **7:** e29151.
2 Huilan S, Zhen LG, Mathan MM, et al. Etiology of acute diarrhoea among children in developing countries: a multicentre study in five countries. *Bull World Health Organ* 1991; **69:** 549–55.
3 Kotloff KL, Nataro JP, Blackwelder WC, 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.
4 Guerrant RL, Oria RB, Moore SR, Oria MOB, Lima AAM. Malnutrition as an enteric infectious disease with long-term effects on child development. *Nutr Rev* 2008; **66:** 487–505.
5 Black RE, Brown KH, Becker S. Effects of diarrhea associated with specific enteropathogens on the growth of children in rural Bangladesh. *Pediatr* 1984; **73:** 799–805.
6 Checkley W, Epstein LD, Gilman RH, Cabrera L, Black RE. Effects of acute diarrhea on linear growth in Peruvian children. *Am J Epidemiol* 2003; **157:** 166–75.
7 Lee G, Yori P, Olortegui MP, et al. Comparative effects of vivax malaria, fever and diarrhoea on child growth. *Int J Epidemiol* 2012; **41:** 531–39.
8 Lorntz B, Soares AM, Moore SR, et al. Early childhood diarrhea predicts impaired school performance. *Pediatr Infect Dis J* 2006; **25:** 513–20.
9 The MAL-ED Network Investigators. 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.
10 Ahmed T, Mahfuz M, Islam MM, et al. The MAL-ED cohort study in Mirpur, Bangladesh. *Clin Infect Dis* 2014; **59** (suppl 4)**:** S280–06.
11 John SM, Thomas RJ, Kaki S, et al. Establishment of the MAL-ED birth cohort study site in Vellore, southern India. *Clin Infect Dis* 2014; **59** (suppl 4)**:** S295–9.
12 Shrestha PS, Shrestha SK, Bodhidatta L, et al. Bhaktapur, Nepal: the MALED birth cohort study in Nepal. *Clin Infect Dis* 2014; **59** (suppl 4)**:** S300–03.
13 Turab A, Soofi SB, Ahmed I, et al. Demographic, socioeconomic, and health characteristics of the MAL-ED network study site in rural Pakistan. *Clin Infect Dis* 2014; **59** (suppl 4)**:** S304–9.
14 Bessong P, Nyathi E, Mahopo C, Netshandama V. Development of the Dzimauli community in Vhembe district, Limpopo province of South Africa for the MAL-ED cohort study. *Clin Infect Dis* 2014; **59** (suppl 4)**:** S317–24.
15 Mduma ER, Gratz J, Patil C, et al. The Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Study (MAL-ED): description of the Tanzanian site. *Clin Infect Dis* 2014; **59** (suppl 4)**:** S325–30.
16 Lima A, Oriá RB, Soares AM, et al. Geography, population, demography, socioeconomic, anthropometry, and environmental status in the MAL-ED cohort and case-control study sites in Fortaleza, Ceará, Brazil. *Clin Infect Dis* 2014; **59** (suppl 4)**:** S287–94.
17 Yori PP, Lee G, Olórtegui MP, et al. Santa clara de nanay: the MAL-ED cohort in Peru. *Clin Infect Dis* 2014; **59** (suppl 4)**:** S310–16.
18 Richard SA, McCormick BJJ, Miller MA, Caulfield LE, Checkley W, MAL-ED Network Investigators. Modeling environmental influences on child growth in the MAL-ED cohort study: opportunities and challenges. *Clin Infect Dis* 2014; **59** (suppl 4)**:** S255–60.
19 Baqui AH, Black RE, Yunus M, Hoque AR, Chowdhury HR, Sack RB. Methodological issues in diarrhoeal diseases epidemiology: definition of diarrhoeal episodes. *Int J Epidemiol* 1991; **20:** 1057–63.
20 Ruuska T, Vesikari T. Rotavirus disease in Finnish children: use of numerical scores for clinical severity of diarrhoeal episodes. *Scand J Infect Dis* 1990; **22:** 259–67.
21 WHO. The Treatment of diarrhoea: a manual for physicians and other senior health workers, 4th revision. Geneva: World Health Organization, 2005. Available at http://whqlibdoc.who.int/publications/2005/9241593180.pdf (accessed Dec 4, 2014).
22 Kosek M, Guerrant RL, Kang G, et al. Assessment of environmental enteropathy in the MAL-ED cohort study: theoretical and analytic framework. *Clin Infect Dis* 2014; **59** (suppl 4)**:** S239–47.
23 Hoest C, Seidman JC, Pan W, et al. Evaluating associations between vaccine response and malnutrition, gut function, and enteric infections in the MAL-ED cohort study: methods and challenges. *Clin Infect Dis* 2014; **59** (suppl 4)**:** S273–79.
24 Richard SA, Barrett LJ, Guerrant RL, Checkley W, Miller MA, MAL-ED Newtork Investigators. Disease surveillance methods used in the 8-site MAL-ED cohort study. *Clin Infect Dis* 2014; **59** (suppl 4)**:** S220–4.
25 Houpt E, Gratz J, Kosek M, et al. Microbiologic methods utilized in the MAL-ED cohort study. *Clin Infect Dis* 2014; **59** (suppl 4)**:** S225–32.
26 Bruzzi P, Green SB, Byar DP, Brinton LA, Schairer C. Estimating the population attributable risk for multiple risk factors using case-control data. *Am J Epidemiol* 1985; **122:** 904–14.
27 Blackwelder WC, Biswas K, Wu Y, et al. Statistical Methods in the Global Enteric Multicenter Study (GEMS). *Clin Infect Dis* 2012; **55** (suppl 4)**:** S246–53.
28 Lehnert-Batar A, Pfahlberg A, Gefeller O. Comparison of confidence intervals for adjusted attributable risk estimates under multinomial sampling. *Biom J* 2006; **48:** 805–19.

29 Højsgaard S, Halekoh U, Yan J. The R Package geepack for Generalized Estimating Equations. *J Stat Software* 2006; **15:** 1–11.

30 Lopman B, Kang G. In praise of birth cohorts: norovirus infection, disease, and immunity. *Clin Infect Dis* 2014; **58:** 492–94.

31 Platts-Mills JA, Liu J, Gratz J, et al. Detection of *Campylobacter* in stool and determination of significance by culture, enzyme immunoassay, and PCR in developing countries. *J Clin Microbiol* 2014; **52:** 1074–80.

32 Ahmed SM, Lopman BA, Levy K. A systematic review and meta-analysis of the global seasonality of norovirus. *PLoS One* 2013; **8:** e75922.

33 Dalton RM, Roman ER, Negredo AA, Wilhelmi ID, Glass RI, Sanchez-Fauquier A. Astrovirus acute gastroenteritis among children in Madrid, Spain. *Pediatr Infect Dis J* 2002; **21:** 1038–41.

34 Walter JE, Mitchell DK. Astrovirus infection in children. *Curr Opin Infect Dis* 2003; **16:** 247–53.

35 Liu J, Kabir F, Manneh J, et al. Development and assessment of molecular diagnostic tests for 15 enteropathogens causing childhood diarrhoea: a multicentre study. *Lancet Infect Dis* 2014; **14:** 716–24.

36 Platts-Mills JA, McCormick BJJ, Kosek M, Pan W, Checkley W, Houpt ER. Methods of Analysis of Enteropathogen Infection in the MAL-ED Cohort Study. *Clin Infect Dis* 2014; **59 (**suppl 4**):** S233–38.