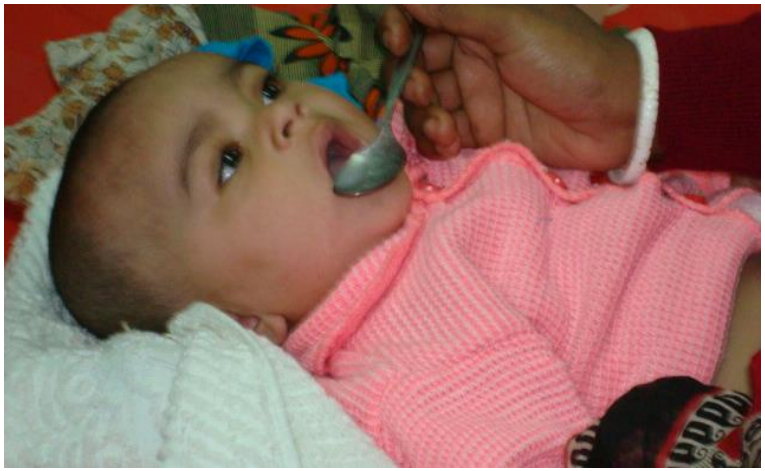
The Global Enterics Multicenter Study (GEMS)



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Fondation Mérieux

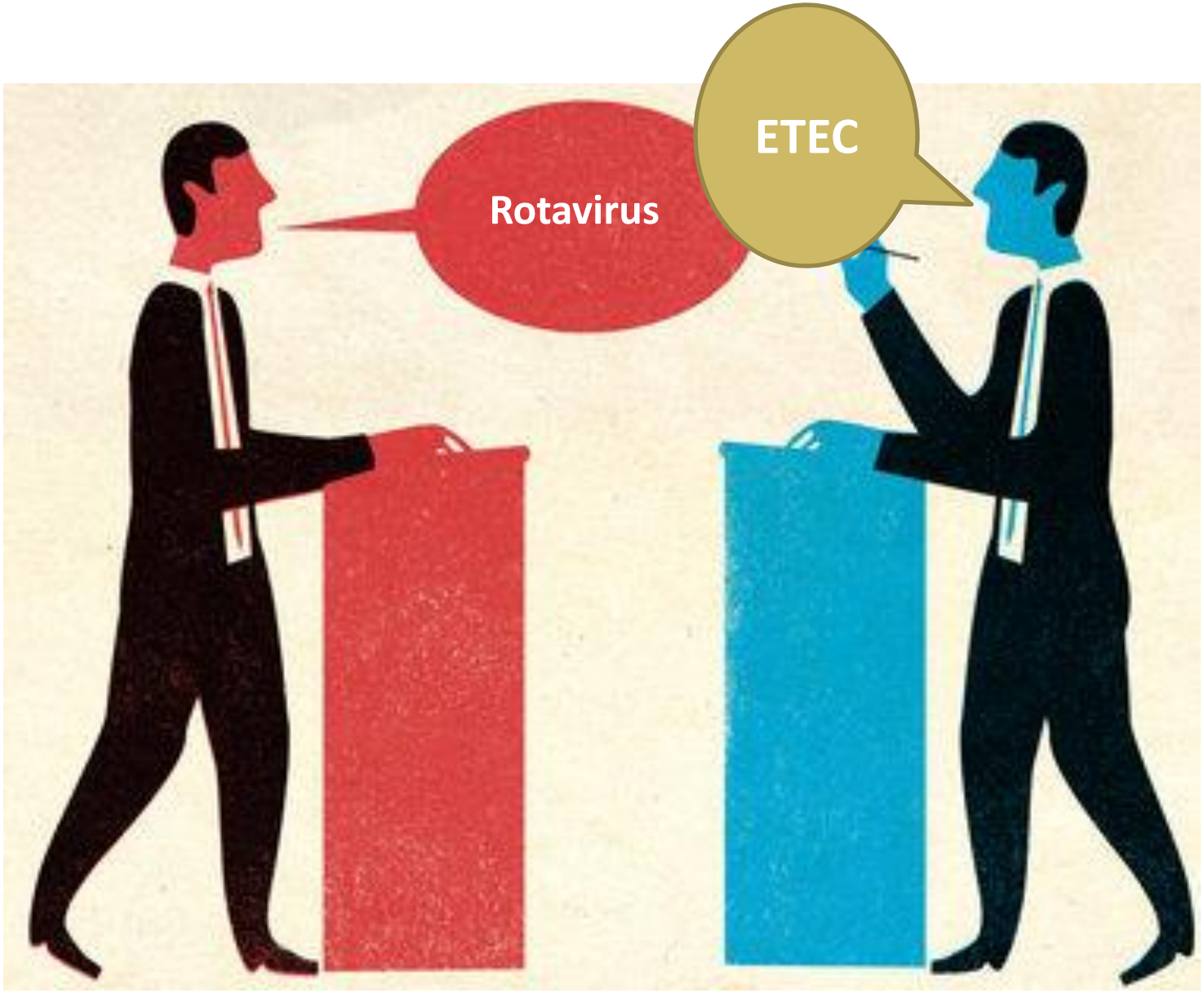
Correlates of Enteric Vaccine-Induced Protection

Annecy, France

21-23 March 2016

UNIVERSITY of MARYLAND
SCHOOL OF MEDICINE
CENTER FOR VACCINE DEVELOPMENT

CVD


Rotavirus
ETEC

Limitations of Previous Studies

Scope

- Few multicenter studies, least data where mortality highest
- Incomplete survey of etiologic agents
- Often limited to children < 24 mos.
- Primarily short surveillance

Methodology

- Case control design not used
- Lack of population-based incidence
- Insensitive microbiological methods
- Strains not characterized
- No follow-up; verbal autopsy data modeled to estimate mortality
- No data to guide vaccine introduction

Primary Objective of GEMS

- Incidence, etiology, and adverse clinical consequences of the most life-threatening and disabling episodes of diarrhea among children < 59 months old living in developing countries in sub-Saharan Africa and S. Asia

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Scope

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GEMS Overview

Multicenter Study

- 7 diverse sites with mod/high U5MR, most severe episodes
- Expanded etiology
- Children 0-59 months of age
- 3 years per site

Methods addressed knowledge gaps

- Case-control study
- Censused population
- Sensitive modern micro assays
- Strains characterized for vaccine devel; strain/specimen repository
- 60-d follow-up : health, vital status
- Economic burden data collected

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PI: Samba Sow, MD MSc

Manhica, Mozambique
PI: Pedro Alonso, MD, PhD

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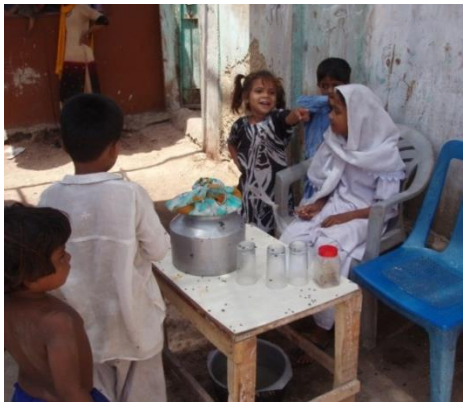

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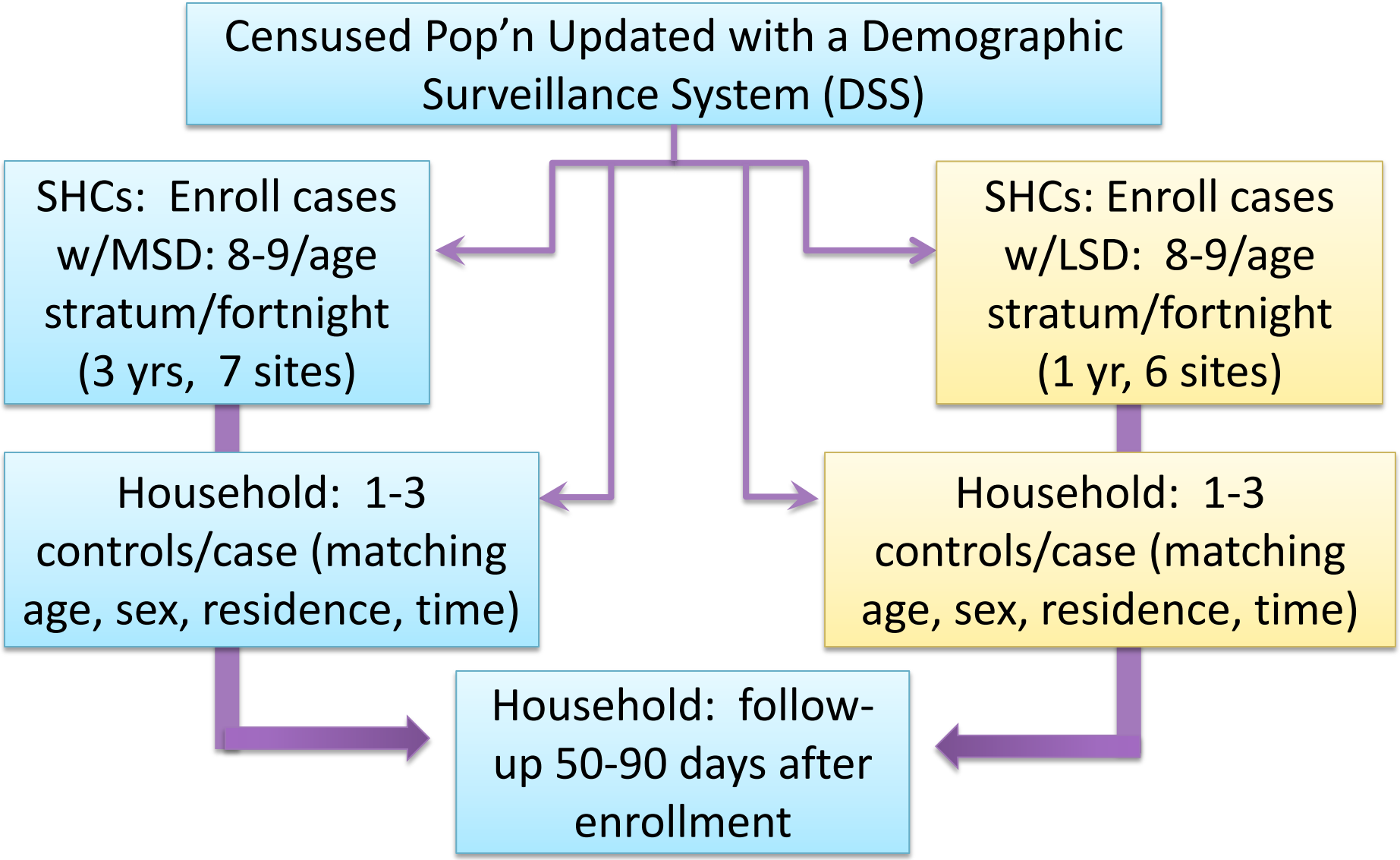
Study Design: Case-Control Study



Deriving Population Based Estimates:

- Health care utilization surveys:

  1,000 randomly selected

  children per DSS round

  – Did child have MSD (LSD) in the previous 7 days; If yes, did they seek care at the SHCs?

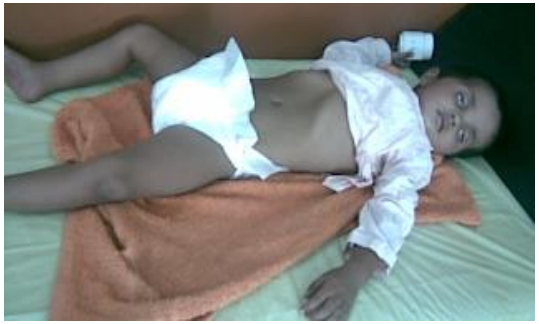
  – Able to measure MSD (LSD) in the SHCs and adjusted the incidence (overall and pathogen-specific) for the % of DSS children who do not seek care at the SHCs for that condition

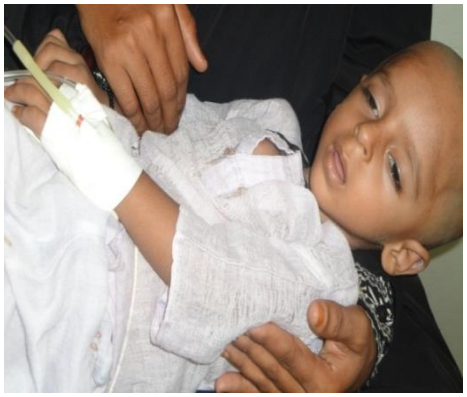
Case eligibility

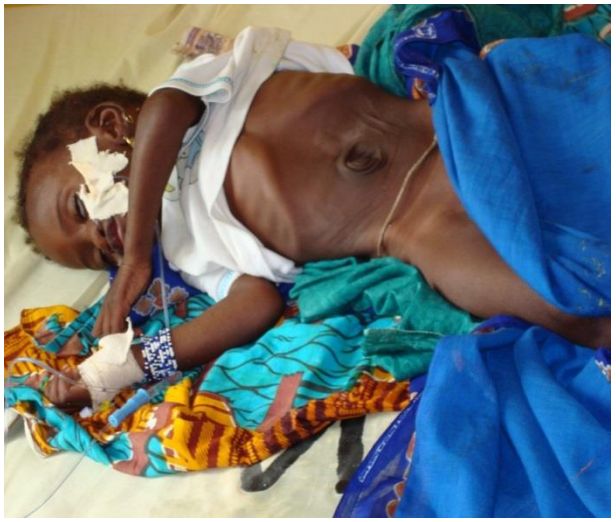
- Documented all visits of children 0-59 months from DSS and asked about presence of diarrhea;
- Evaluated each for eligibility

- Children 0-59 months (3 age strata: 0-11 mo., 12-23 mo., 24-59 mo.)
- In DSS population, Seeking care at SHC
- Diarrhea (≥3 abnormally loose stools in previous 24h)
- New episode (after at least 7 diarrhea-free days)
- Acute (onset within the previous 7 days)
  - MSD case definition (≥1 of the following)
    - Sunken eyes (more than normal per parent);
    - Decreased skin turgor;
    - IV hydration administered or prescribed;
    - Dysentery (visible blood in a loose stool); or
    - Hospitalized with diarrhea or dysentery
  - LSD case definition: all others

Children with MSD in GEMS







Data collection (Cases & Controls)

- **Enrollment**
  - Data
    - Demographic, Clinical, Epidemiologic
    - Anthropometrics
  - Stool sample: either
    - Whole stool (≥3g or 3 ml)* within 12 h of registration at center, before antibiotics, OR
    - Rectal swab, prior to antibiotics, followed by whole stool within 12 h of registration at center

- **60-day follow-up visit to the home**
  - Clinical sequelae; death
  - Nutritional outcome (anthropometrics)
  - Direct observation of environmental risks

*Only whole stool collected from controls

Laboratory Analysis*

- Culture
  - *Salmonella*
  - *Shigella*
  - *Campylobacter*
  - *Aeromonas*
  - *Vibrio cholerae*
- Multiplex PCR:
  - ETEC, EPEC, EAEC, STEC
- Immunoassay:
  - Rotavirus
  - Enteric adenovirus 40 and 41
- RT-PCR
  - Norovirus (genotypes I and II)
  - Sapovirus
  - Astrovirus
- Immunoassay
  - *Giardia lamblia*
  - *Entamoeba histolytica*
  - *Cryptosporidium* spp.
- Post hoc
  - ETEC CFA (by PCR and monoclonals)
  - EAEC AAF typing
  - Rotavirus typing
  - *Cryptosporidium, Giardia* speciation
  - TAQMan Reanalysis
- GEMS 1a
  - Toxigenic *B. fragilis, C. difficile, H. pylori,* intestinal helminths

*All assays except Post hoc performed at the sites

Attributable Fraction (AF)

- Takes into account the OR/RR, the prevalence of the pathogen, and, using the Bruzzi model, interactions/ confounding due to co-infecting pathogens
- Proportion of diarrhea cases over a specified time that would be prevented by eliminating exposure to a pathogen (assuming exposures are causal)

ENROLLMENT

487,386
child-years of observation contributed by children 0-59 months in DSS

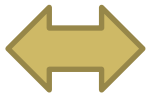
626,519
visits to SHC

66,009 (11% of visits)
to SHC were for diarrhea

14,753 (22% of diarrhea visits)
met criteria for MSD

9,980 (68% of eligible)
were invited to participate

9,439 cases (95% of invited)
enrolled and analyzable



13,129 matched controls

Key Findings

1. MSD Etiology:
   - Conventional methods
   - Additional disease burden from LSD
   - New insights using TAQMan
   - How strain characterization informs vaccine development
2. Impact of MSD on linear growth
   - Pathogens associated with linear growth faltering
3. Impact of MSD on mortality
   - Relationship between stunting and survival following an episode of MSD
   - Pathogens associated with mortality

Key Findings – #1. MSD Etiology :

5 pathogens accounted for ~50% of AF

- Rotavirus
- *Cryptosporidium*
- *Shigella*
- ST-producing ETEC
- To a lesser extent Adenovirus 40/41

- *Campylobacter, Aeromonas, and V. cholerae* had regional importance

Adjusted Attributable Fraction, 0-11 mo.

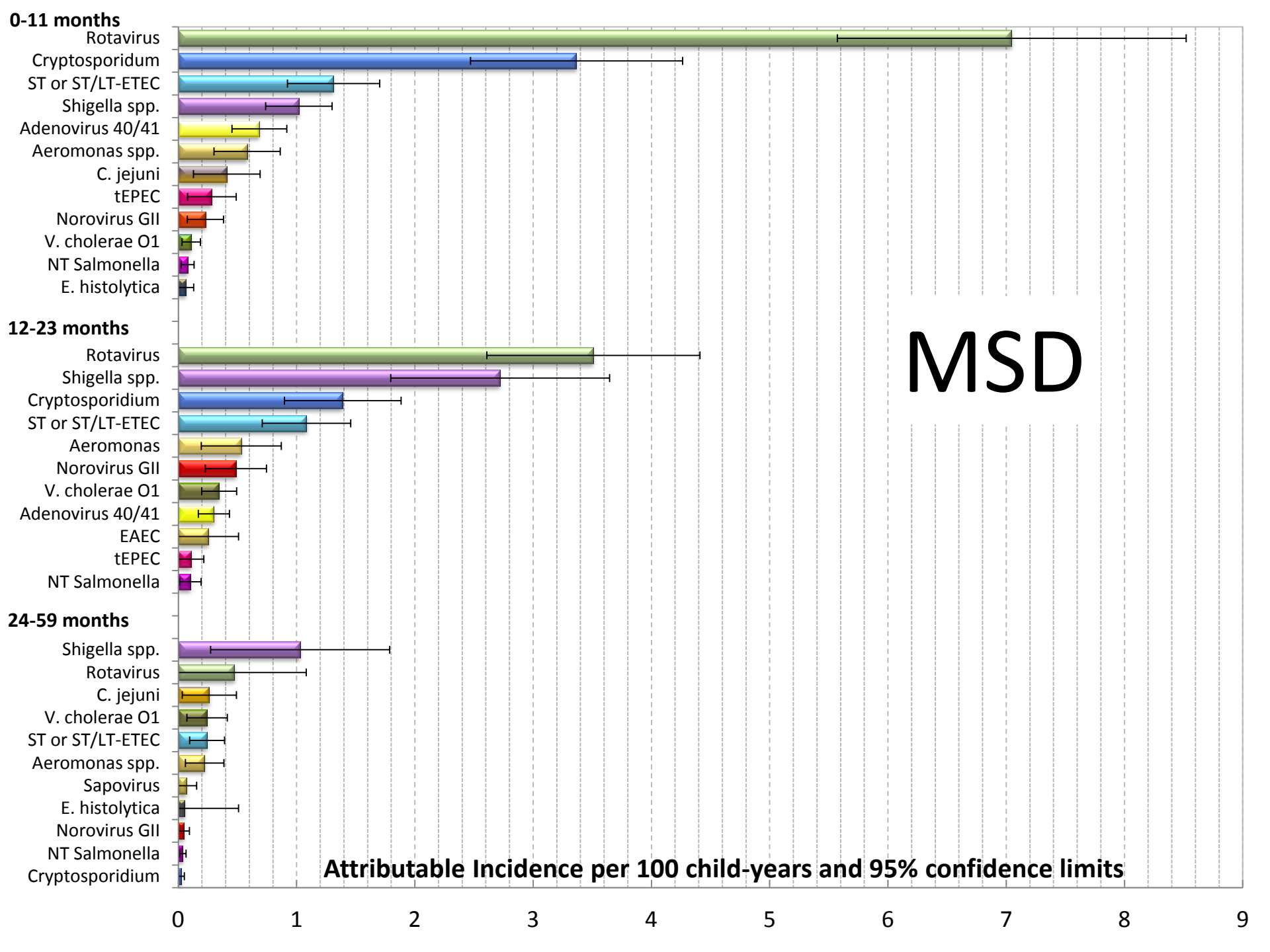
	Gamb	Kenya	Mali	Mozamb	Banglad	India	Pakista
No. cases	400	673	727	374	550	672	633
Rotavirus	23.1	18.9	20.6	32.4	17.0	28.2	23.7
Cryptosporidium	11.4	9.3	13.8	14.1	5.7	12.4	4.9
ST-ETEC (ST-only or LT/ST)	4.5	6.4	3.9	3.0	1.4	3.1	7.4
Shigella	3.6	4.2	-	-	13.0	2.0	6.8
Norovirus GII	7.1	-	-	-	-	-	-
Aeromonas	-	-	-	-	9.9	-	10.3
Adenovirus 40/41	2.2	1.9	1.8	1.8	3.8		1.9
C. jejuni	-	-	-	-	9.9	4.0	6.3
tEPEC	-	4.9	-	-	-	-	-
NT Salmonella	-	-	-	-	3.9	-	-
Astrovirus	-	-	-	-	-	-	3.0
V. cholerae O1	-	-	-	-	-	-	3.1
E. Histolytica	-	-	-	-	3.5	-	-

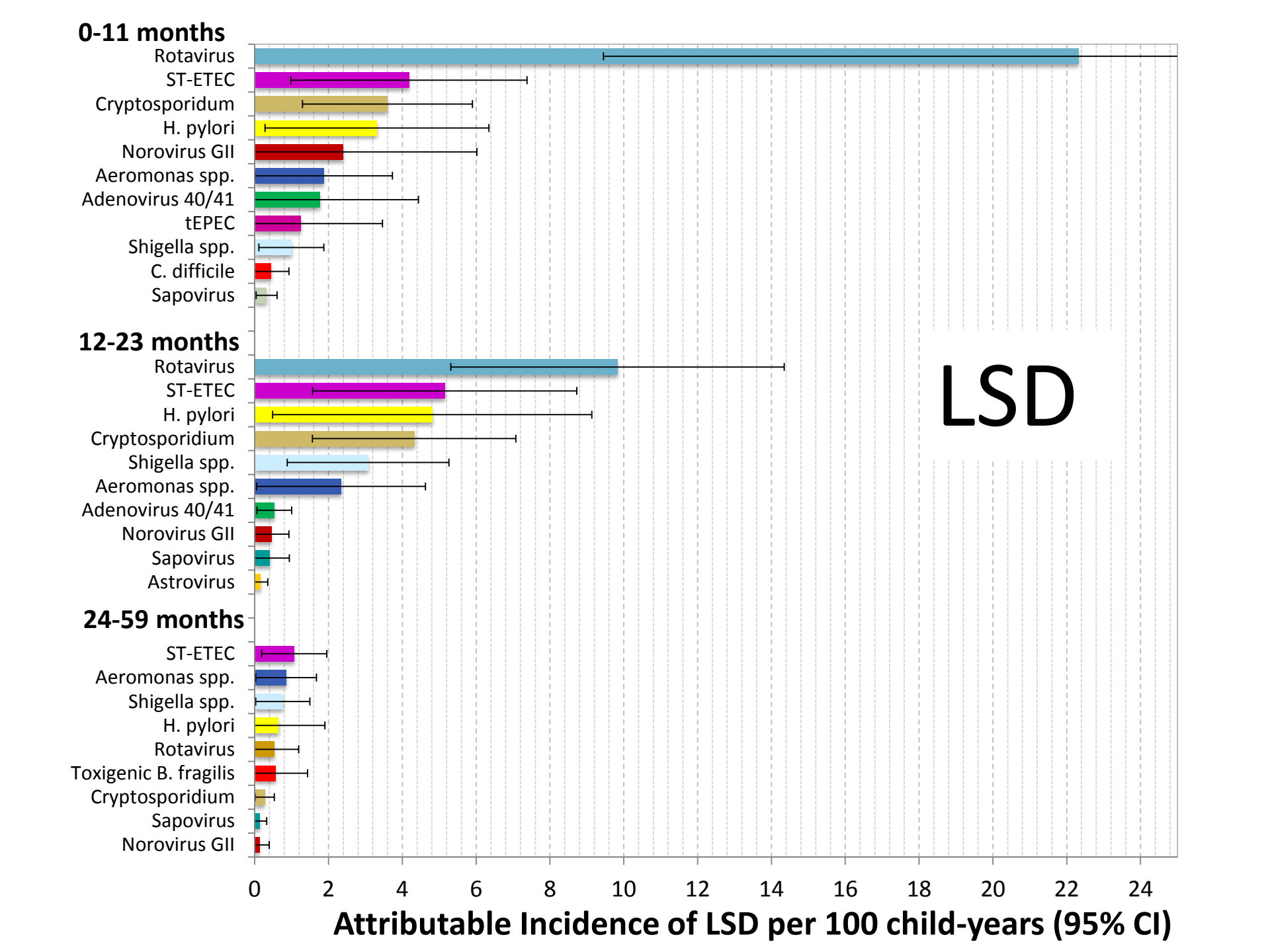
Adjusted Attributable Fraction, 12-23 mo.

	Gamb	Kenya	Mali	Mozamb	Banglad	India	Pakist
No. of cases	N=455	N=410	N=682	N=195	N=476	N=588	N=399
Rotavirus	17.2	13.8	11.7	5.2	17.4	24.5	10.1
Shigella	11.8	4.5	2.2	5.9	52.9	6.3	11.5
Cryptosporidium	7.7	8.4	4.1	9.5	-	8.8	7.6
ST-ETEC (ST-only or LT/ST)	5.6	7.1	2.7	9.4	-	5.9	5.8
Aeromonas	-	-	-	-	11.4	-	9.6
Norovirus GII	8.4	-	-	-	-	5.1	-
V. cholerae O1	-	-	-	-	1.5	3.2	7.6
EAEC	-	-	-	-	9.3	-	-
Adenovirus 40/41	2.3	-	-	-	-	4.5	-
NT Salmonella	-	3.7	-	-	-	-	-
E. histolytica	-	1.3	-	-	-	1.9	-
tEPEC	-	3.4	-	-	-	-	-
C. jejuni	-	-	-	2.0	-	-	-

Adjusted Attributable Fraction, 24-59 mo.

	Gamb	Kenya	Mali	Mozamb	Banglad	India	Pakista
No. of cases	174	393	624	112	368	308	226
Shigella	12.6	9.0	1.8	16.8	69.3	11.2	8.6
Aeromonas	-	-	-	-	15.3	-	24.6
Rotavirus	12.8	3.3	3.1	-	-	14.2	-
V. cholerae O1	-	-	-	7.6	2.7	8.2	13.1
ST-ETEC (ST-only or LT/ST)	8.4	4.7	-	-	-	7.0	6.0
C. jejuni	-	-	-	-	-	9.5	12.1
E. histolytica	-	-	2.2	-	-	2.0	-
Norovirus GII	7.7	-	-	-	-	-	-
NT Salmonella	-	3.7	-	-	1.7	-	-
Cryptosporidium		2.6	-	-	-	-	-
Sapovirus	-	-	-	-	-	3.8	-


0-11 months
Rotavirus
Cryptosporidum
ST or ST/LT-ETEC
Shigella spp.
Adenovirus 40/41
Aeromonas spp.
C. jejuni
tEPEC
Norovirus GII
V. cholerae O1
NT Salmonella
E. histolytica
12-23 months
Rotavirus
Shigella spp.
Cryptosporidium
ST or ST/LT-ETEC
Aeromonas
Norovirus GII
V. cholerae O1
Adenovirus 40/41
EAEC
tEPEC
NT Salmonella
24-59 months
Shigella spp.
Rotavirus
C. jejuni
V. cholerae O1
ST or ST/LT-ETEC
Aeromonas spp.
Sapovirus
E. histolytica
Norovirus GII
NT Salmonella
Cryptosporidium
MSD
Attributable Incidence per 100 child-years and 95% confidence limits
0
1
2
3
4
5
6
7
8
9


0-11 months
Rotavirus
ST-ETEC
Cryptosporidum
H. pylori
Norovirus GII
Aeromonas spp.
Adenovirus 40/41
tEPEC
Shigella spp.
C. difficile
Sapovirus
12-23 months
Rotavirus
ST-ETEC
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Cryptosporidium
Shigella spp.
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Norovirus GII
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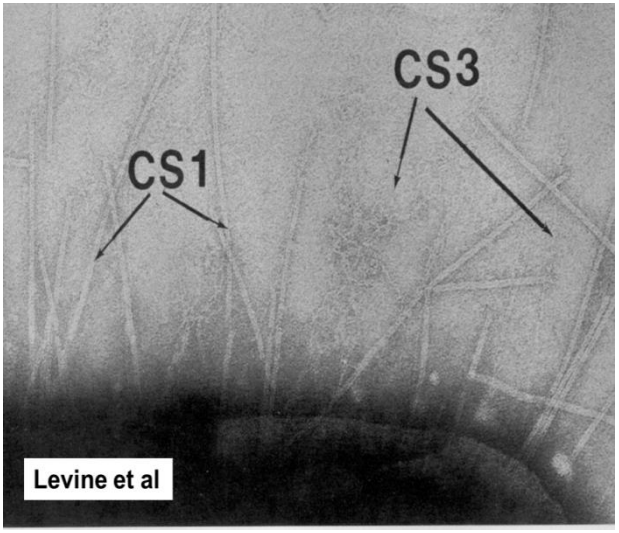
Astrovirus
24-59 months
ST-ETEC
Aeromonas spp.
Shigella spp.
H. pylori
Rotavirus
Toxigenic B. fragilis
Cryptosporidium
Sapovirus
Norovirus GII
LSD
0
2
4
6
8
10
12
14
16
18
20
22
24
Attributable Incidence of LSD per 100 child-years (95% CI)

Strain Characterization Informs Vaccine Development

Prevalence of *S. sonnei* & *S. flexneri* serogroups and Proposed Vaccine Component Serotypes among *Shigella* Isolates from GEMS (Livio, Levine, et al CID 2014; 59:933-41)

Serogroup, serotype, or sub-serotype	Year 1	Year 2	Year 3
Total isolates	**N=457**	**N=345**	**N=328**
S. sonnei	21%	22%	30%
S. flexneri 2a, 3a, 6	40%	42%	40%
S. sonnei + *S. flexneri* 2a, 3a, 6	61%	64%	70%
S. sonnei + all *S. flexneri* serotypes other than S. flex 7a	88%	86%	89%

Prevalence of the main CFAs among GEMS case ETEC isolates



Toxin profile	Case isolates (N=857)	CFA/I, CS1, CS2, CS3, CS4, CS5, CS6
LT	279	23%
ST	310	66%
LT/ST	268	74%
ST+LT/ST	578	70%

#2 – Impact of MSD on Linear Growth Faltering

- In general, the Height for Age Z score (HAZ) at enrollment for cases was not significantly different from the HAZ for matched controls
- At the follow-up visit 2-3 months after an episode of MSD
  - Cases had significantly more growth faltering (negative ΔHAZ) compared to matched controls, adjusting for enrollment HAZ and time to follow-up
  - Significantly more infants were stunted

	Enrollment	Follow-up
None	50.7%	41.5%
Mild	30.8%	32.1%
Moderate	12.8%	18.1%
Severe	5.8%	8.4%

Diarrheal pathogens associated with linear growth faltering among MSD cases

- Association with growth faltering was statistically significant for:
  - *0-11 m: Cryptosporidium*, tEPEC and non-typhoidal *Salmonella*
  - *12-23 m:* ST-ETEC, *Cryptosporidium,* rotavirus and *V. cholerae*
  - 24-59 m: *V. cholerae*
- Infants and toddlers: *Shigella* was associated with growth faltering when WHO-recommended antibiotics for dysentery were not prescribed
- Toddlers: Prescription of WHO-recommended antibiotics was associated with improved HAZ

#3- MSD-related Mortality

Mortality: Single episode of MSD increases risk of death 8.5-fold over the ensuing ~ 60 days after the initial episode, across all sites

- **When:** 61% of deaths occurred > 7 days after enrollment; 33% of deaths occurred > 21 days after enrollment
- **Where:** 44% of cases died at a medical facility; 56% of cases died at home or outside a medical facility

Site-specific Mortality Data

	Cases			Controls			Odds ratio
	Total	Died	% Died	Total	Died	% Died	(95% CI)
Mozamb	681	51	7.5%	1296	11	0.9%	13.4 (6.1-29.3)*
The Gambia	1029	39	3.8%	1569	7	0.6%	7.0 (3.0-16.5)*
Kenya	1476	52	3.5%	1883	11	0.5%	5.5 (2.8-10.7)*
Mali	2033	23	1.1%	2064	5	0.2%	5.5 (1.8-16.5)*
Pakistan	1258	16	1.3%	1838	1	0.05%	13.1 (.99-172)#
Bangla	1394	7	0.5%	2465	1	0.04%	12.4 (2.0-77.5)*
India	1568	2	0.1%	2014	1	0.05%	2.6 (0.34-19.6)$
All sites	**9439**	**190**	**2.0%**	**13129**	**37**	**0.3%**	**8.5 (5.8-12.5)***

* p< 0.01; # p=0.051 $ p=NS

Association between enrollment HAZ and risk of death during FU

- Proportional hazard (Cox) regression models for all sites combined, adjusted for enrollment age, site, and days of follow-up
- The risk of death increased with more severe stunting
  - **Among children with MSD,** for every 0.5 decrease in enrollment HAZ, the estimated hazard of death increased by 30%, 15%, and 46% in the 0-11, 12-23, and 24-59 month strata, respectively

Weighted adjusted hazards ratios by pathogen and risk of death between enrollment and follow-up visit in cases of MSD

	Pathogen present		Pathogen absent		Adjusted hazard ratio (HR)	
	Total	**% deaths**	**Total**	**% deaths**	**HR (95% CI)**	**p**
0-11 months						
Typical EPEC	375	6·4%	3654	2·3%	2·6 (1·6–4·1)	<0·001
ST-ETEC	256	4·7%	3773	2·5%	1·9 (0·99–3·5)	0·05
12-23 months						
Crypto	374	4·0%	2830	1·6%	2·3 (1·3–4·3)	0·006

Summary and Conclusions

- **Burden:** a small number contributed most attributable moderate-to-severe diarrhea cases: rotavirus, *Cryptosporidium*, ST-ETEC, and *Shigella*, and, to a lesser extent, adenovirus 40/41.
- **Outcome 2-3 months after illness onset:**
  - Significantly more linear growth faltering among cases than controls after a single episode of MSD
  - 8.5 fold higher mortality among cases than controls after a single episode of MSD
- **Implications:** targeted interventions for a limited no. of pathogens might have a substantial impact

Next Step: Vaccine Impact on Diarrhea in Africa (VIDA)

- Burden, etiology, and adverse clinical consequences of MSD in 3 GEMS countries in sub-Saharan Africa that have introduced rotavirus vaccine
- Mali, The Gambia, and Kenya

Acknowledgments

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