Diarrheal Diseases in Asia: overview and update

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Overview of the talk

- **Global and Regional burden of diarrheal disease**
- **Emerging trends in the etiology of diarrheal pathogens**
- **New Frontiers**
  - Asymptomatic infections
  - Intestinal microbiota and diarrhoel pathogens
  - Polymicrobial infections
- **Interventions for diarrheal diseases**
- **Conclusion**

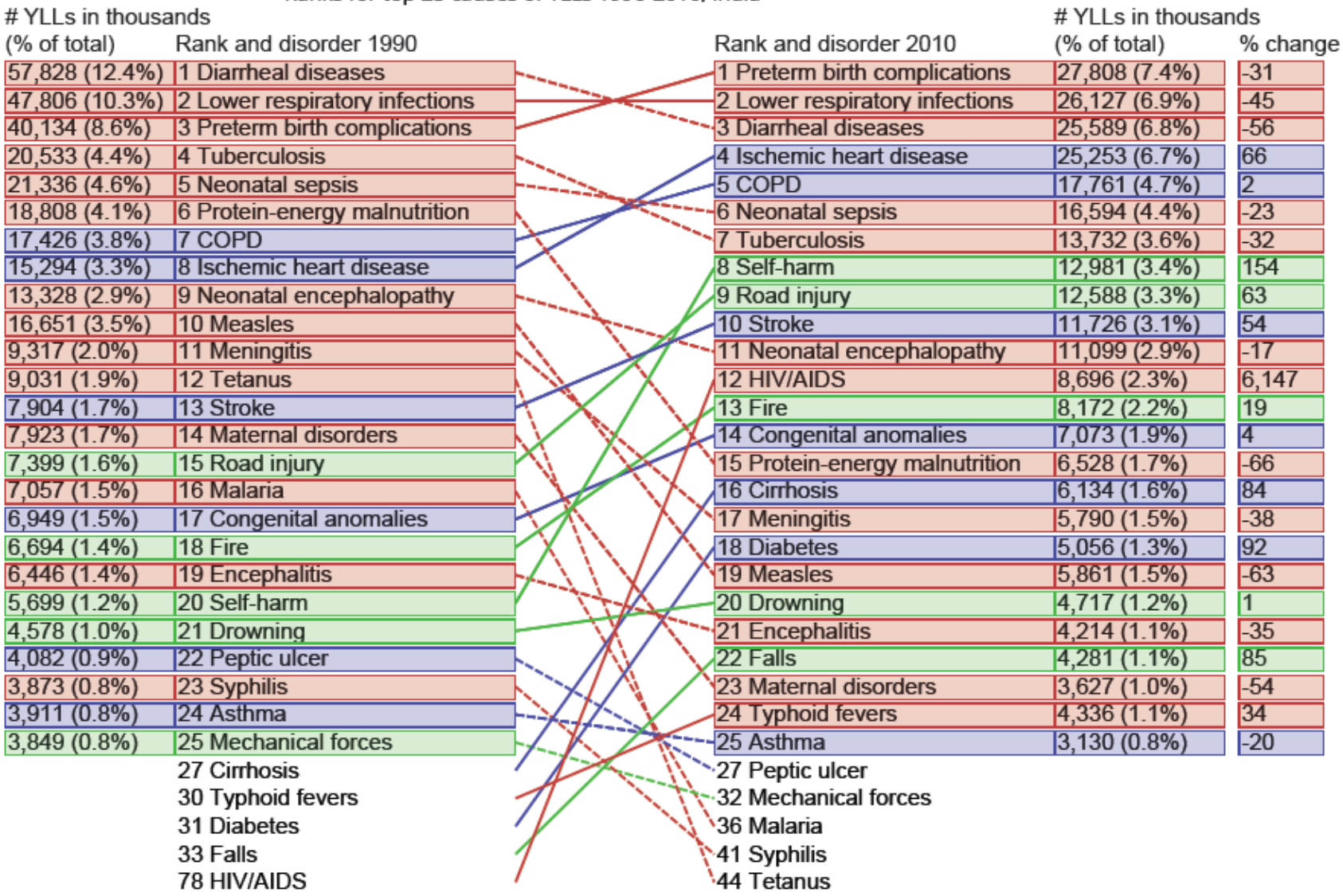
Global and Regional burden of diarrheal diseases

Top 25 causes of Years of Life Lost due to premature mortality from 1990 to 2010

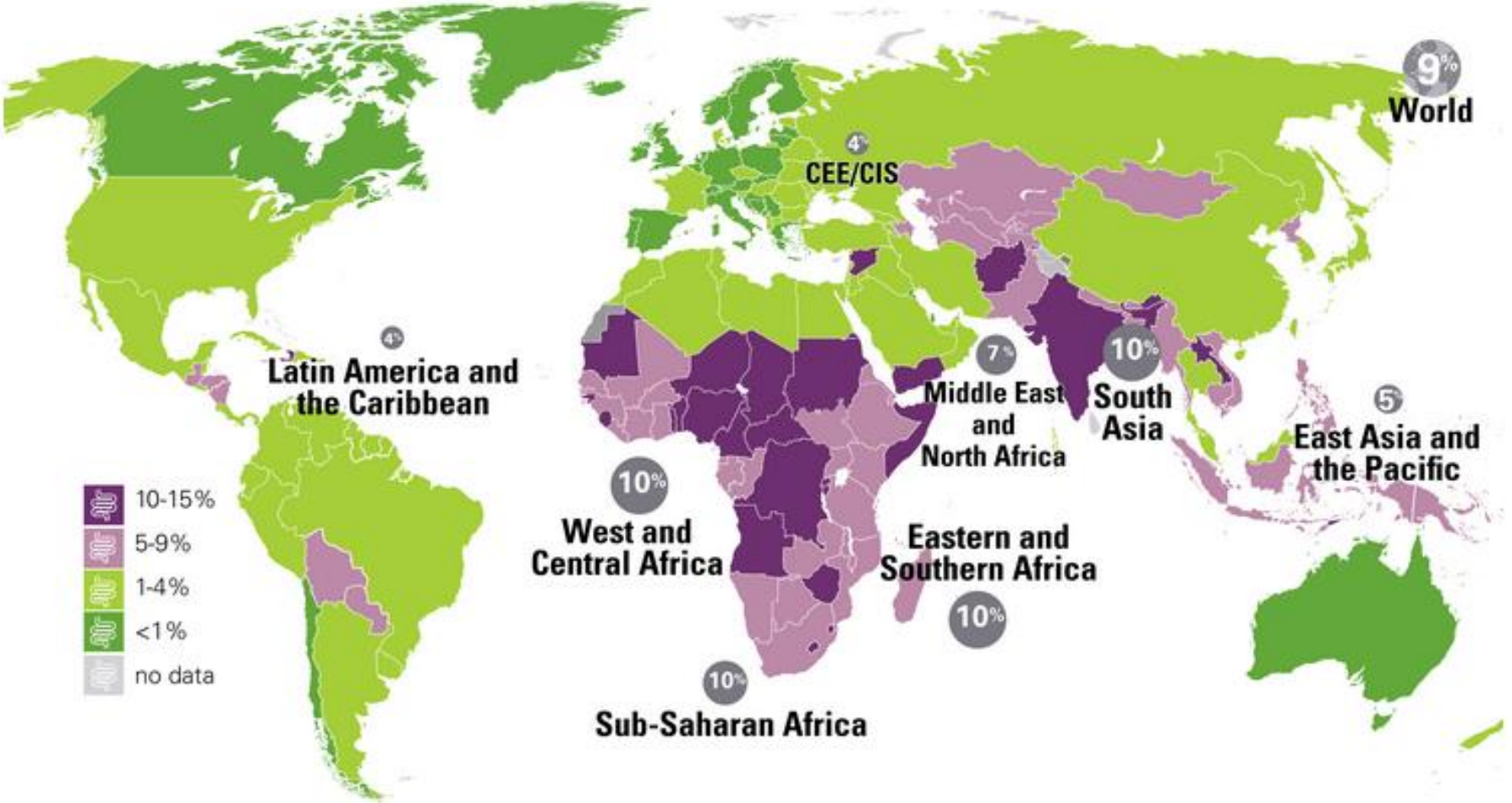
CAUSES OF PREMATURE DEATH

Years of life lost (YLLs) quantify premature mortality by weighting younger deaths more than older deaths.

Ranks for top 25 causes of YLLs 1990-2010, India



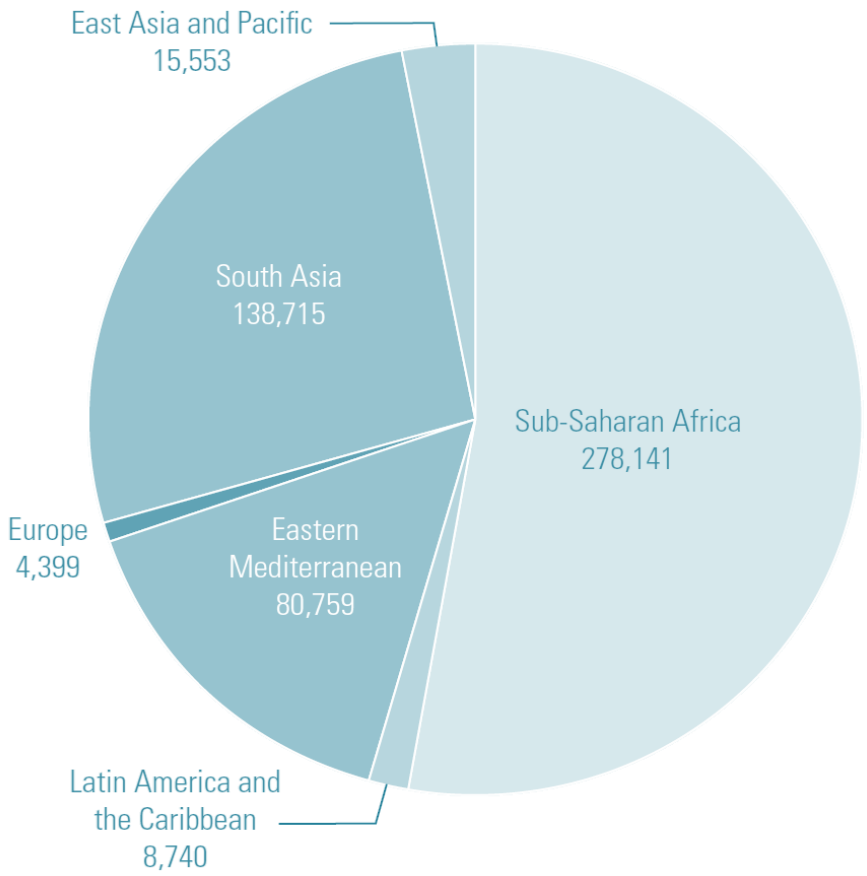
Percentage of deaths among children under age 5 attributable to diarrhea, 2015



Diarrhea remains a leading killer of young children, despite the availability of simple treatment solutions

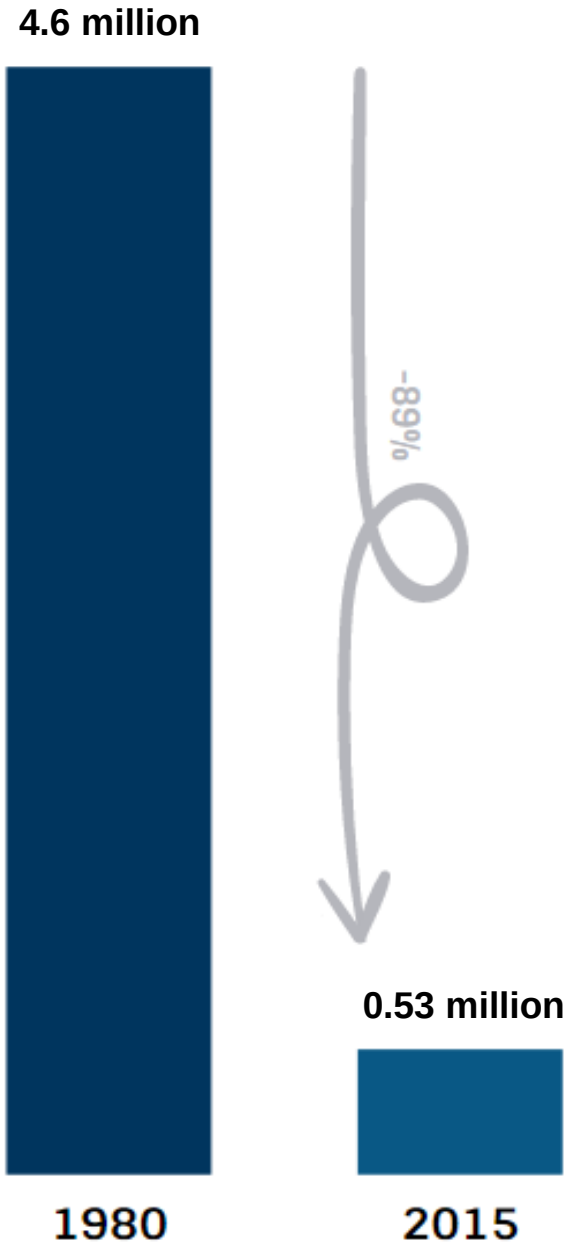
Source: WHO and Maternal and Child Epidemiology Estimation Group (MCEE) provisional estimates 2015

Regional Burden of Diarrhea Mortality, Ages 0–4 Years, 2015



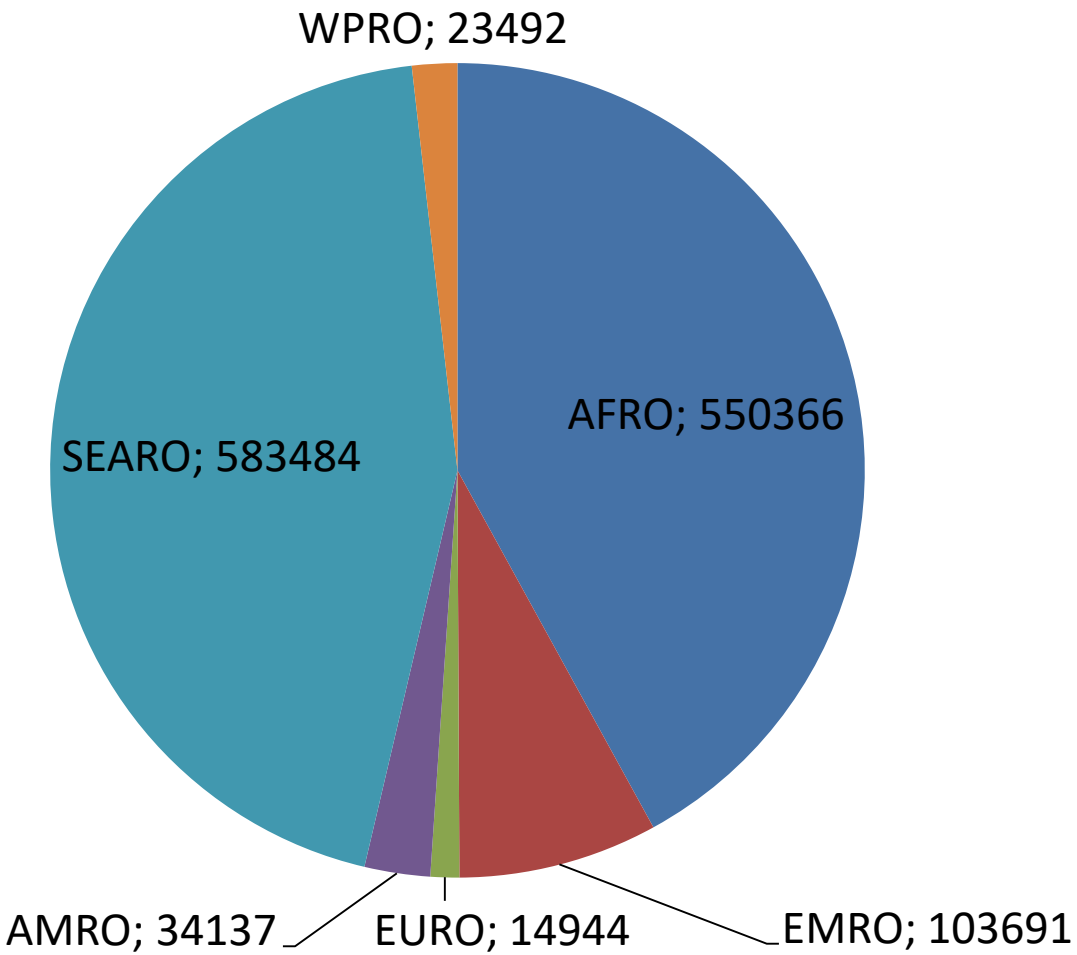
- Estimated number of deaths due to diarrhea 526,000
- 89% drop from 1980 and a striking 58% from 2000 to 2015
- Sub-Saharan Africa and South Asia account for 90% of the total
- 72% of the diarrhea deaths occur in the first two years of life

Keusch *et al* 2016; Liu, Hill, and others 2016



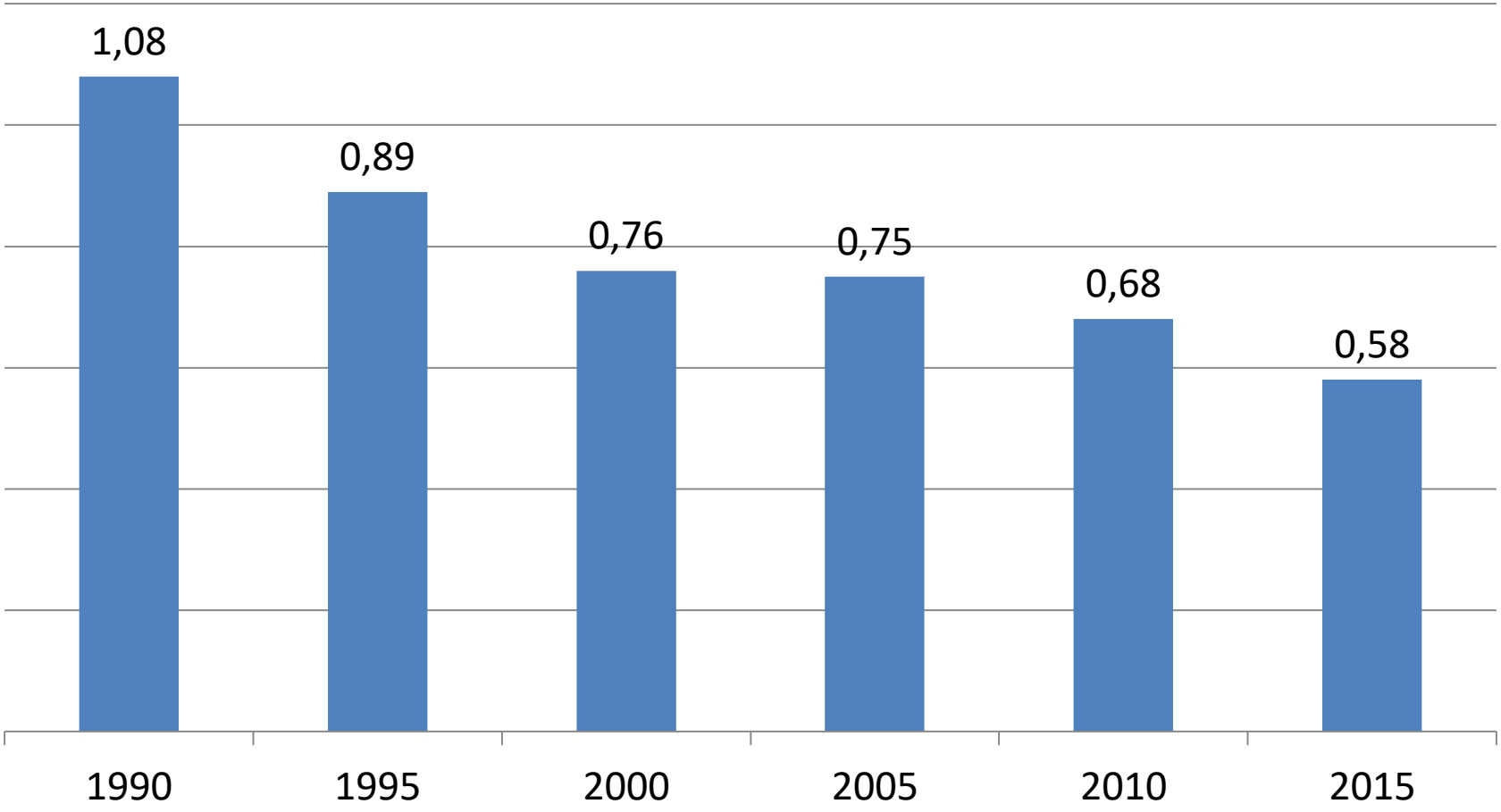
Annual number of deaths from diarrheal diseases among the 0–4 year age group in low- and middle-income countries (LMICs)

Deaths due to Diarrheal Diseases in WHO Regions



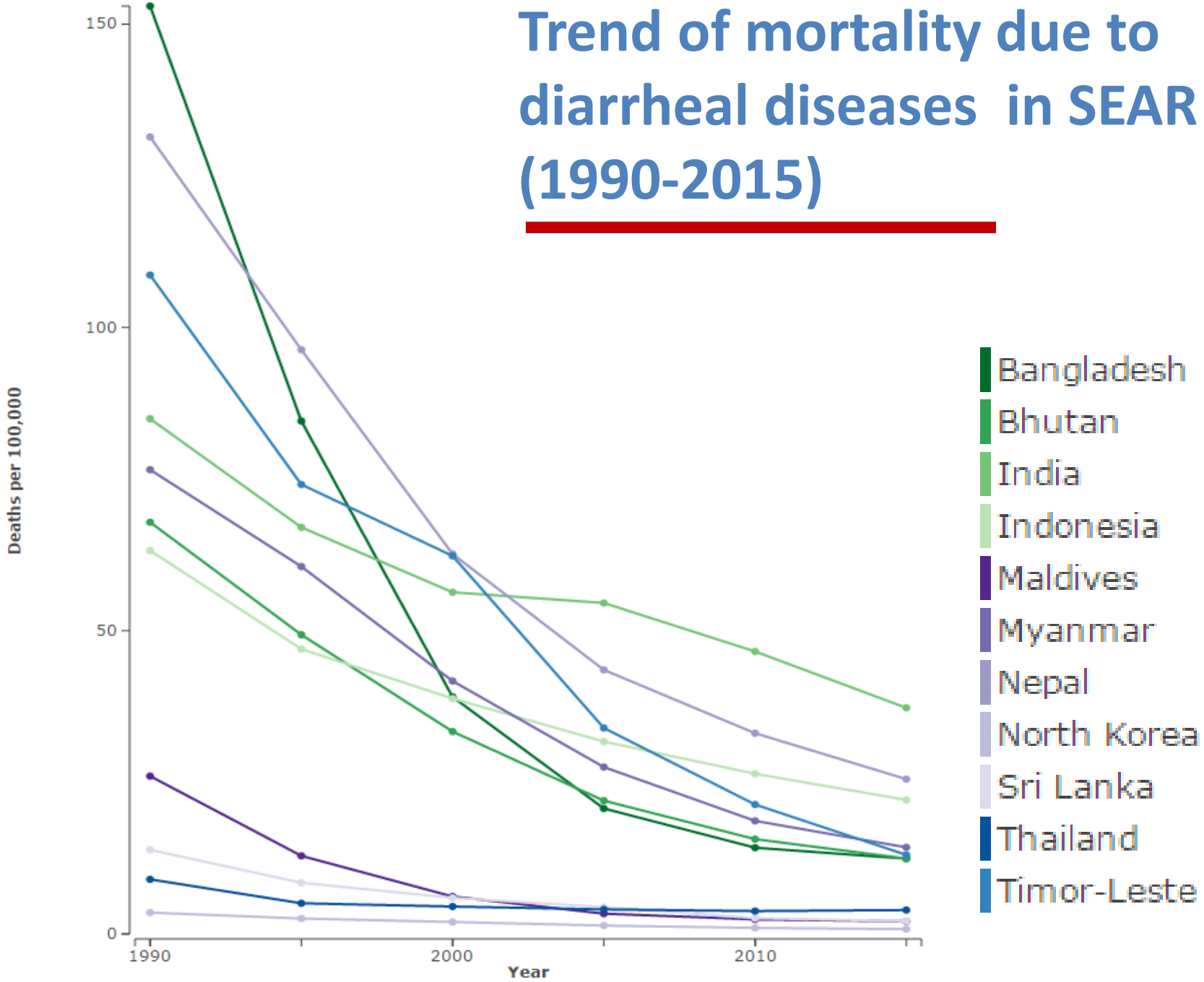
Source: Institute for Health Metrics and Evaluation. GBD 2015 © 2017 University of Washington. https://vizhub.healthdata.org/gbd-compare/

Deaths due to Diarrheal Diseases in SEAR (in millions)



Source: Institute for Health Metrics and Evaluation. GBD 2015 © 2017 University of Washington. https://vizhub.healthdata.org/gbd-compare/

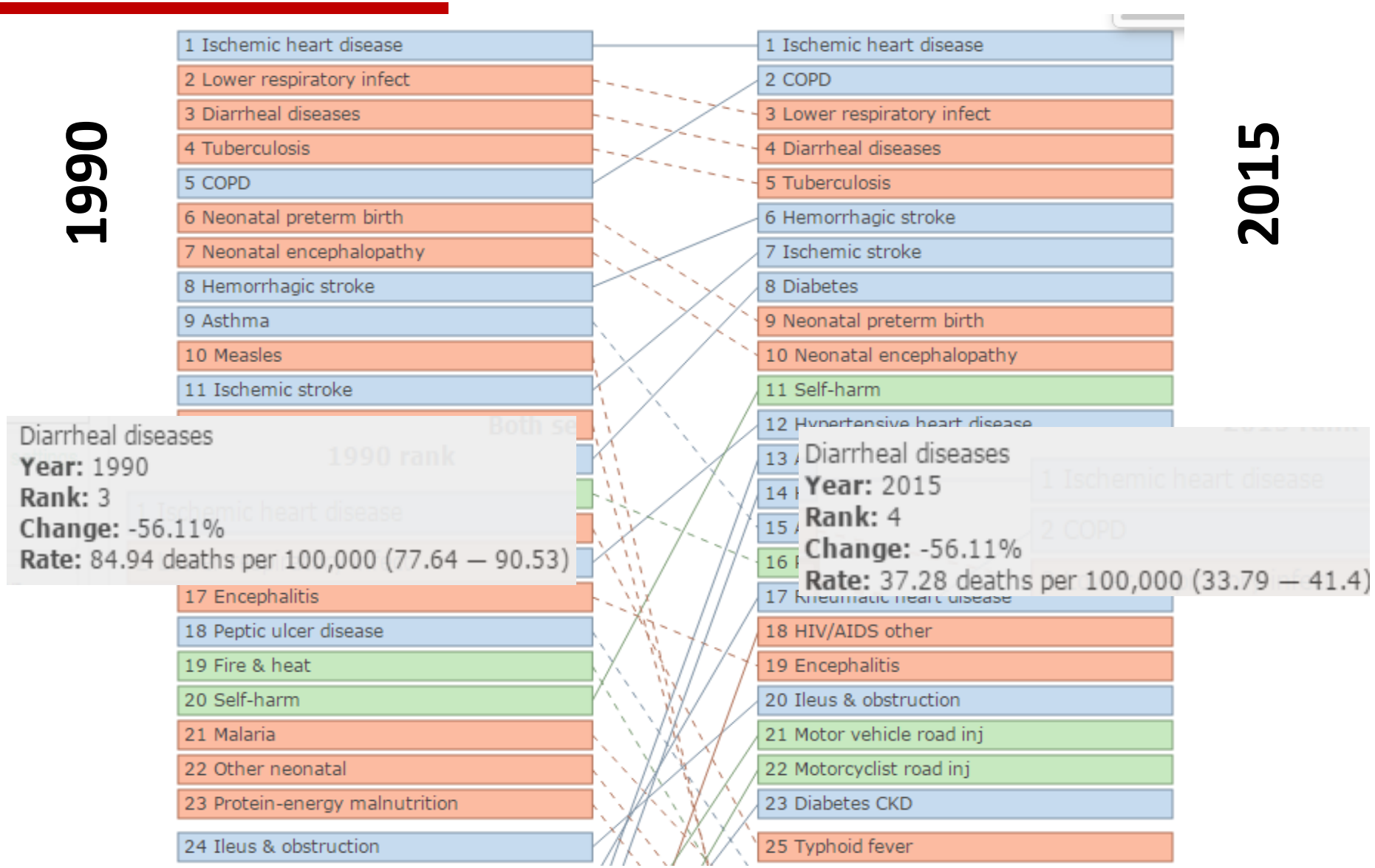
Trend of mortality due to diarrheal diseases in SEAR (1990-2015)



Major interventions in Diarrheal diseases

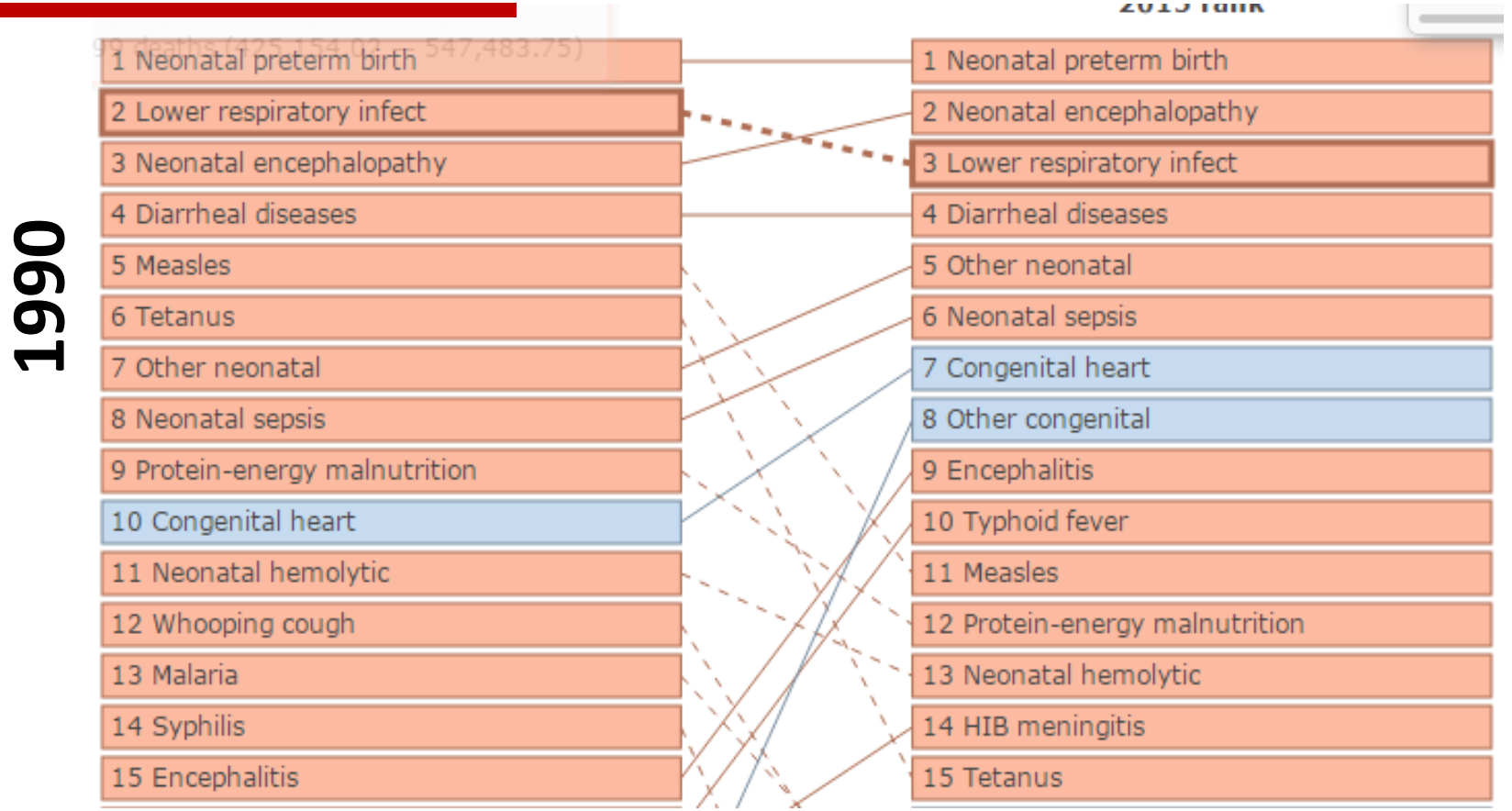
- **Early use of Oral rehydration solutions**
- **Appropriate use of antibiotics for bloody diarrhea and dysentery**
- **Continued breast feeding**
- **Nutritional interventions for persistent diarrhea**
- **Rapid restoration of nutritional status in all diarrhea patients**

Major killers in India



Source: Institute for Health Metrics and Evaluation. GBD 2015 © 2017 University of Washington. https://vizhub.healthdata.org/gbd-compare/

Major killers in under 5 children in India

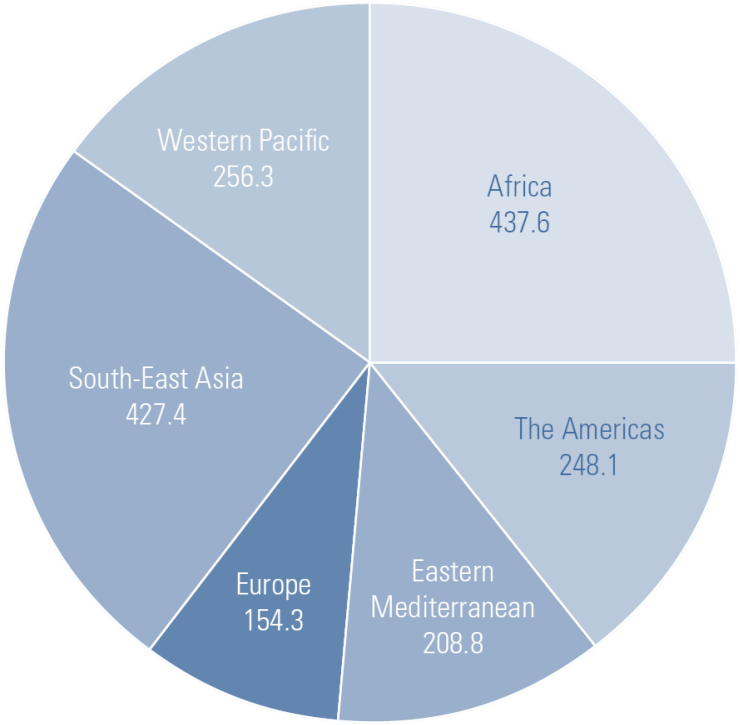


Diarrheal diseases
Year: 1990
Rank: 4
Change: -69.66%
Rate: 277.68 deaths per 100,000 (232.87 – 312.01)

Diarrheal diseases
Year: 2015
Rank: 4
Change: -69.66%
Rate: 84.24 deaths per 100,000 (72.07 – 98.52)

Source: Institute for Health Metrics and Evaluation. GBD 2015 © 2017 University of Washington. https://vizhub.healthdata.org/gbd-compare/

Regional Burden of Diarrhea, Ages 0–4 Years, 2010

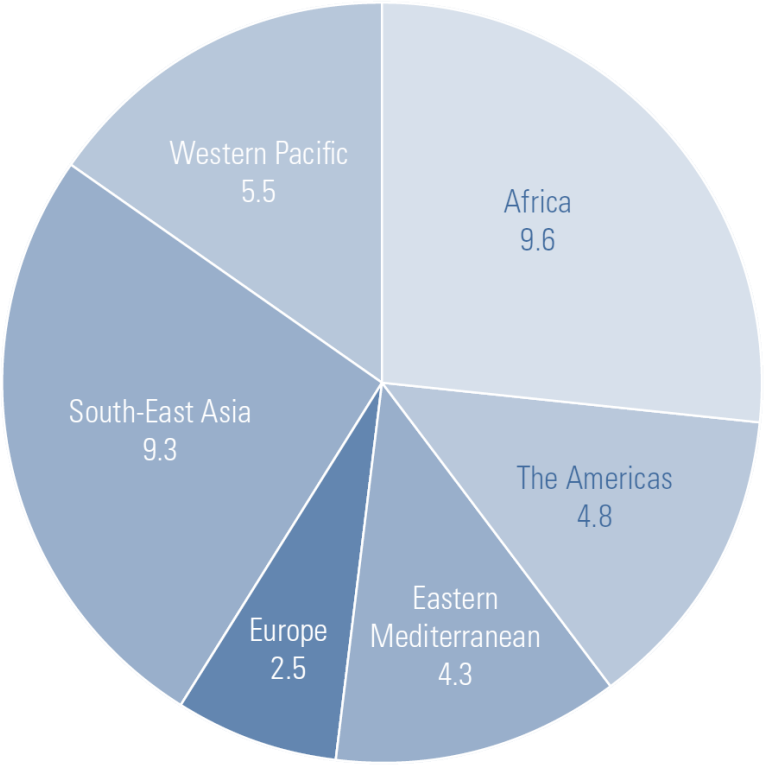


- Estimated global diarrhea incidence rates have not changed significantly since 1980
- Children in Sub Saharan Africa and South Asia experience an average of 2.7 episodes of diarrhea per year
- Most mild to moderate lasting an avg . of 4.3 days

- Incidence rates vary but are higher in children in LIC and LMIC countries and highest in Sub-Saharan Africa (3.3 episodes per child per year)

Fischer Walker and others 2013; Keusch *et al* 2016;

Regional Burden of Severe Diarrhea Episodes, Ages 0–4 Years, 2010



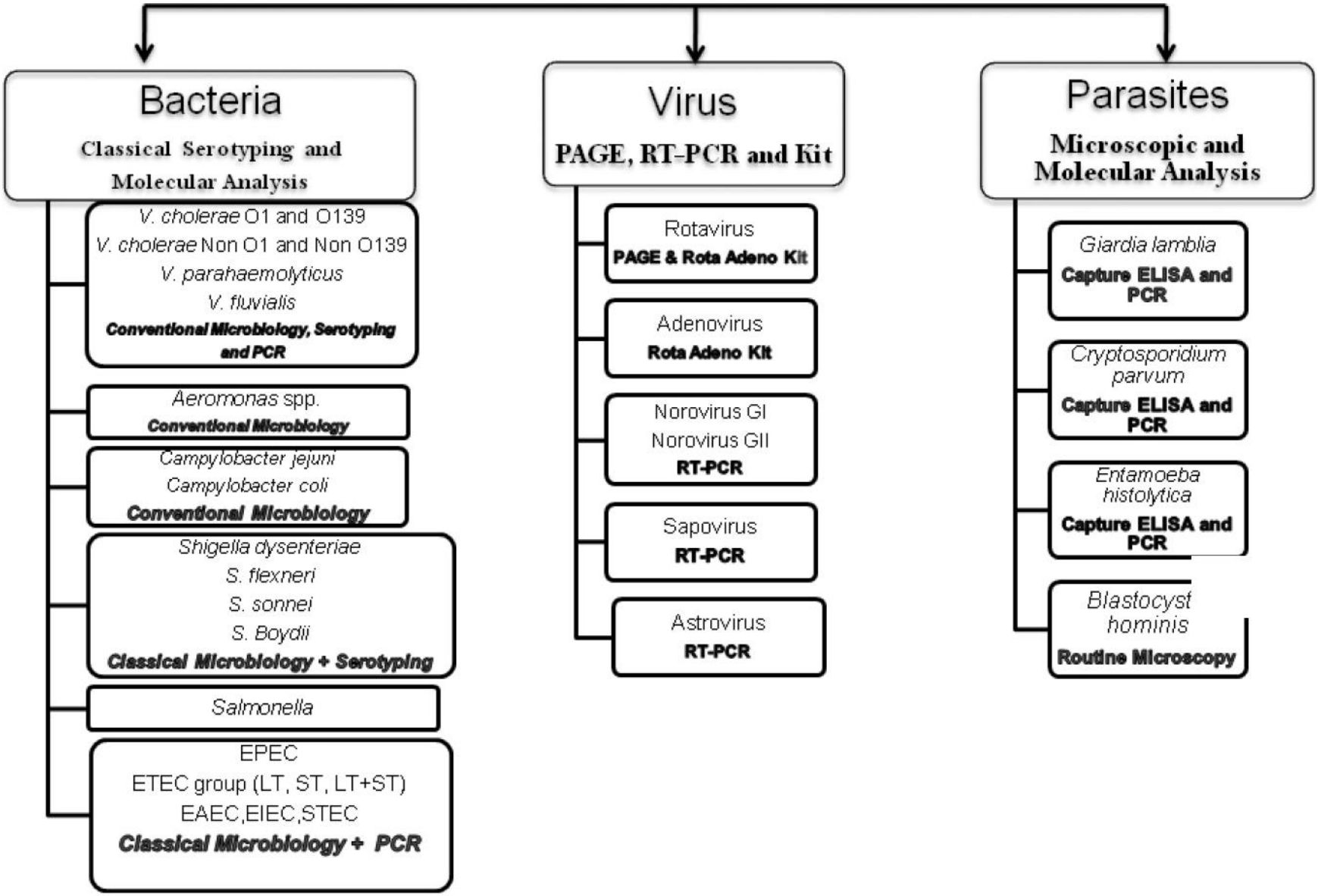
- 5 to 15% of watery diarrhea cases progress to persistent diarrhea
- More than 50 percent of severe episodes occur in Sub Saharan Africa and South-East Asia

Fischer Walker and others 2013; Keusch *et al* 2016;

Emerging trends in the etiology of diarrheal pathogens

Many bacterial, viral and parasitic etiologies cause diarrheal disease but only a few account for a major portion of the burden

Bacterial Viral and Parasitic diarrhoeal pathogens

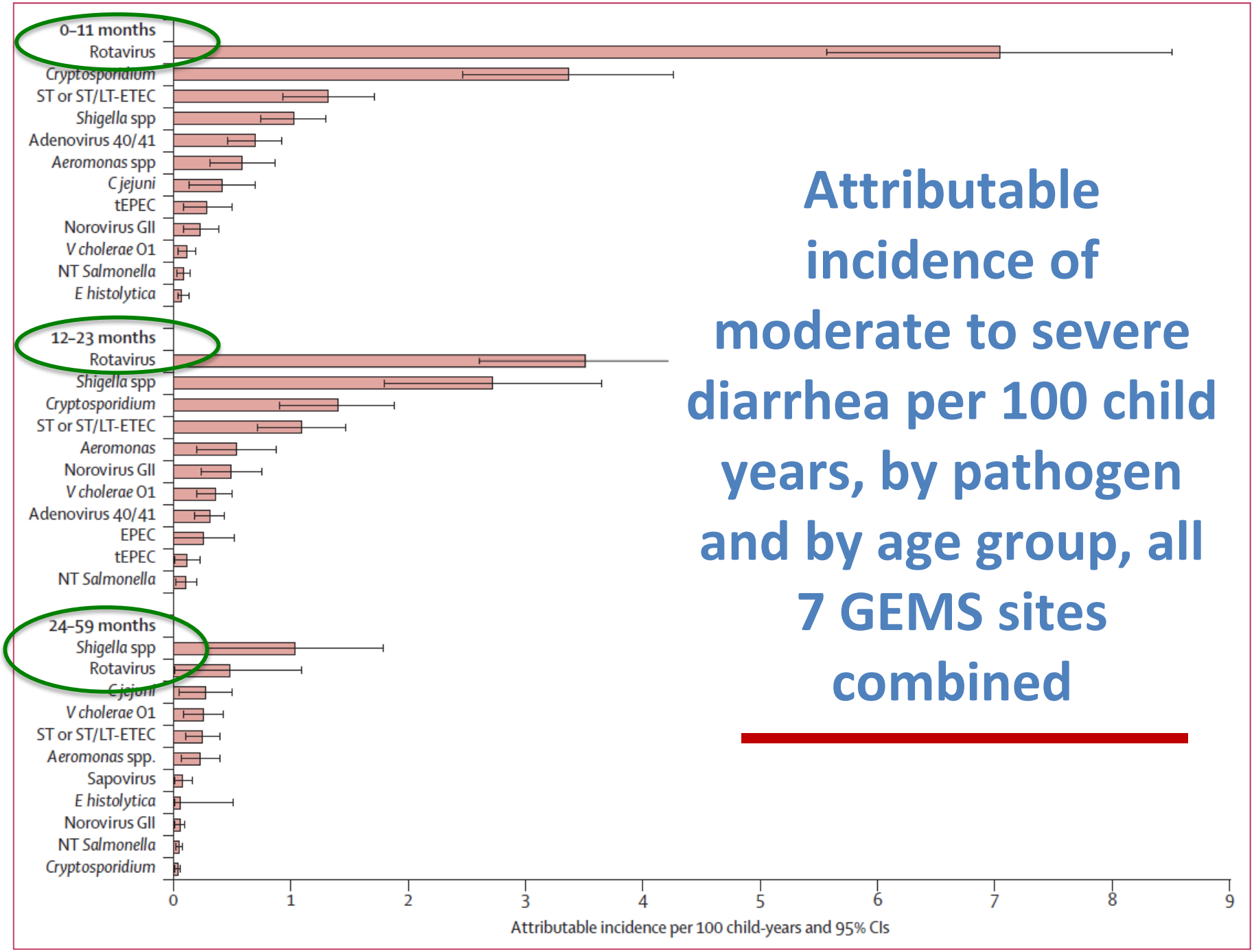


Diarrheal Etiologies

- In one study, 40 % of cause-specific attributable diarrhea mortality was due to two organisms: rotavirus (27.8%) and EPEC (11.1%) (Lanata and others 2013)
- Another large, multisite, clinic-based prospective case-control study of children under age five years with MSD identified four pathogens – rotavirus, Cryptosporidium, ETEC and Shigella responsible for most attributable episodes of MSD (Kotloff et al 2013)

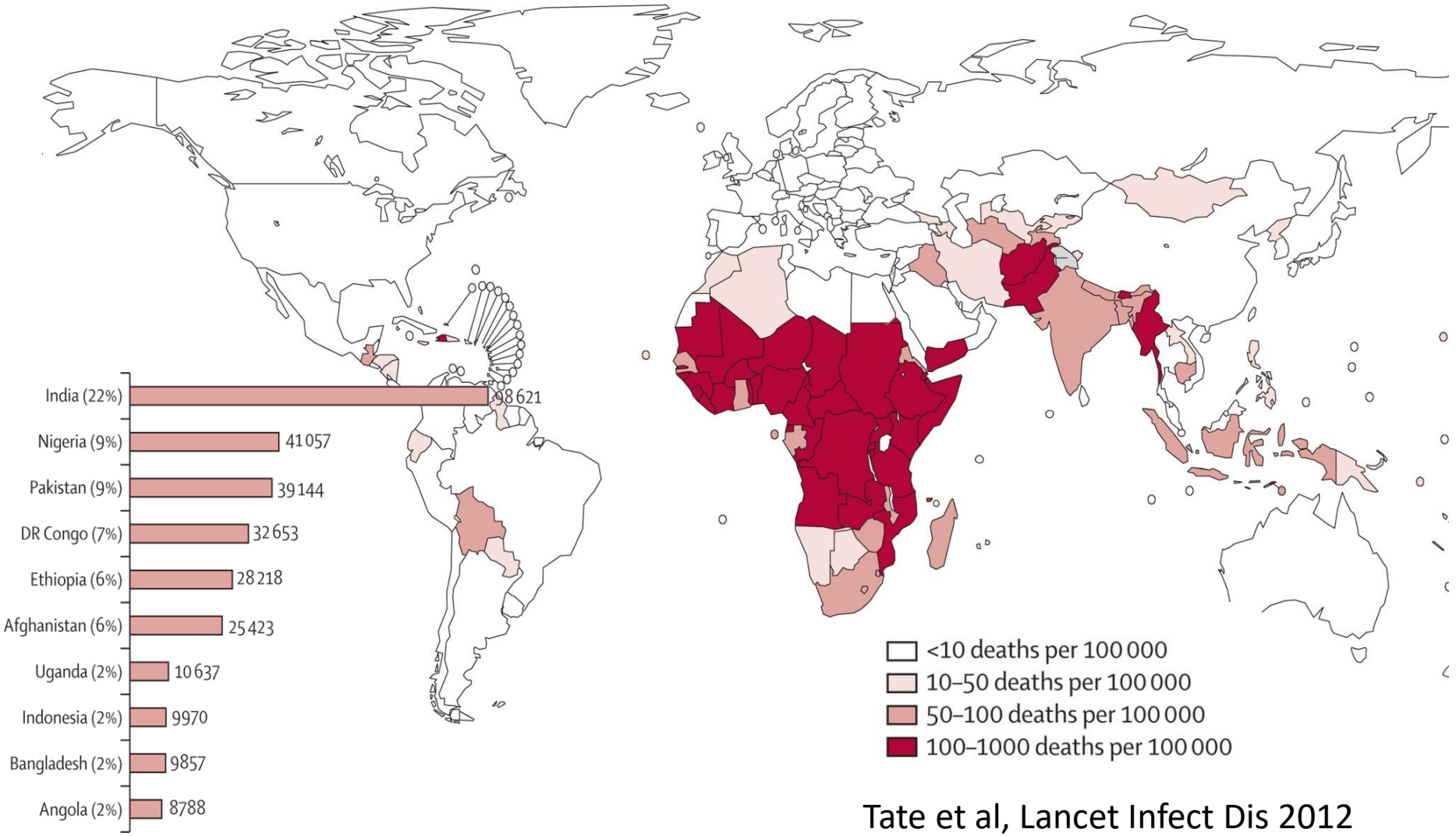
Diarrheal Etiologies

- Certain pathogens such as Rotavirus, Shigella, *Vibrio cholerae* and Adenovirus serotypes 40/ 41 were more commonly isolated in children with moderate to severe illness.
- 72% of controls without diarrhoea also harbored one or more putative pathogens and 31% had two or more reflecting the fecally contaminated environment in which they live.



Attributable incidence of moderate to severe diarrhea per 100 child years, by pathogen and by age group, all 7 GEMS sites combined

Rotavirus deaths estimates for 2008



Tate et al, Lancet Infect Dis 2012

Nair *et al. Gut Pathogens* 2010, **2**:4
http://www.gutpathogens.com/content/2/1/4

Gut Pathogens

RESEARCH Open Access

Emerging trends in the etiology of enteric pathogens as evidenced from an active surveillance of hospitalized diarrhoeal patients in Kolkata, India

Gopinath Balakrish Nair*[1], Thandavarayan Ramamurthy[1], Mihir Kumar Bhattacharya[1], Triveni Krishnan[1], Sandipan Ganguly[1], Dhira Rani Saha[1], Krishnan Rajendran[1], Byomkesh Manna[1], Mrinmoy Ghosh[2], Keinosuke Okamoto[3] and Yoshifumi Takeda[4]

Age wise isolation of enteric pathogens

(Nov 2007 to Oct 2009)

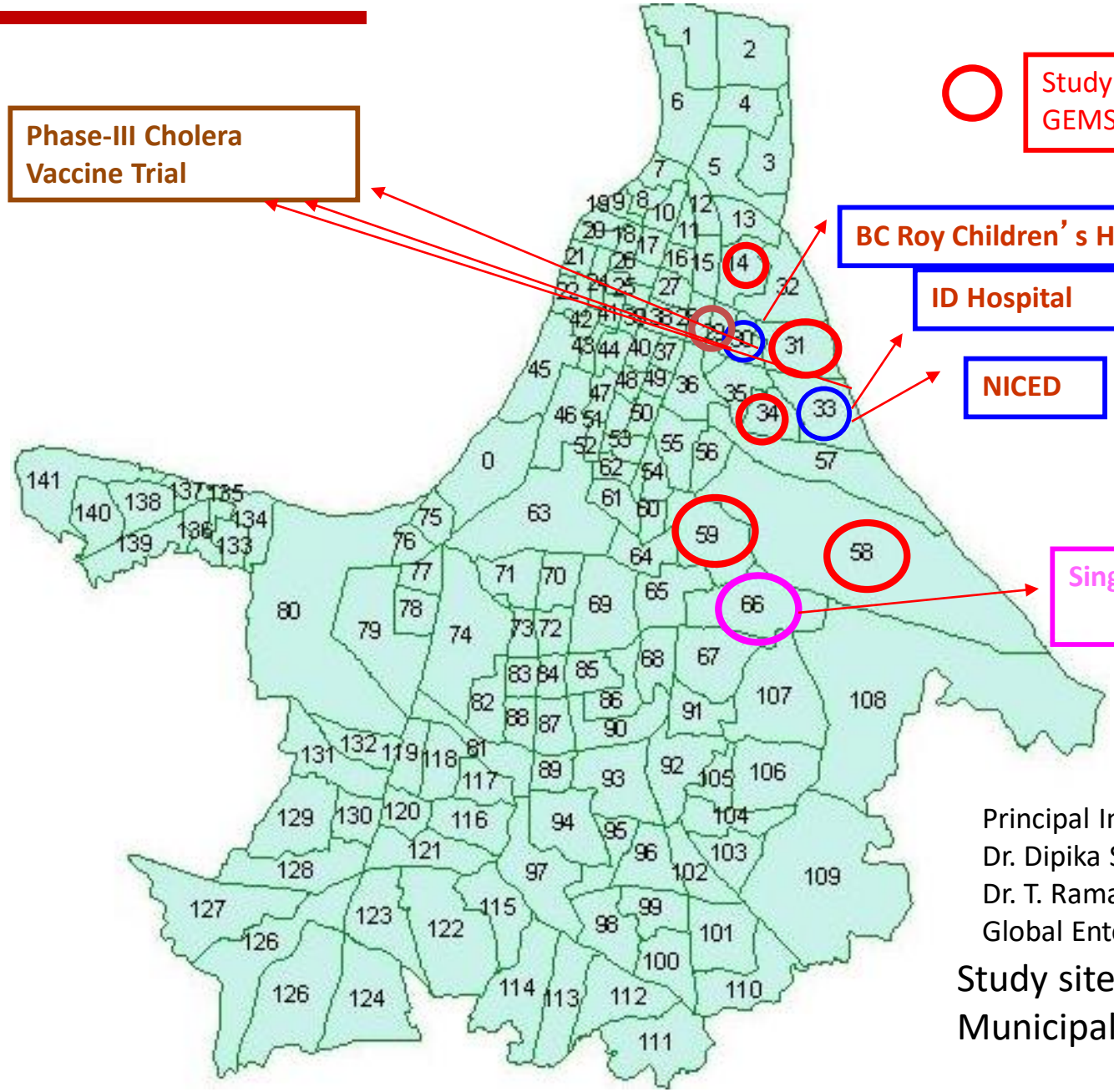
Pathogen	Age <5 years				Age ≥ 5 yr (n=1871)	All Age Group (n=2519)
	0 - 11 month (n=245)	12 - 23 month (n=227)	24 - 59 month (n=176)	Total Age <5 yr (n=648)		
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Bacteria						
Vibrio cholerae O1	22(9)	34(15)	50(28.4)	106(16.4)	548(29.3)	654(26)
Vibrio cholerae O139	0	0	0	0	2(0.1)	2(0.1)
Vibrio cholerae Non O1 Non O139	2(0.8)	1(0.4)	1(0.6)	4(0.6)	51(2.7)	55(2.2)
V. parahaemolyticus	1(0.4)	0	2(1.1)	3(0.5)	71(3.8)	74(2.9)
Vibrio fluvialis	3(1.2)	7(3.1)	1(0.6)	11(1.7)	44(2.4)	55(2.2)
Aeromonas spp.	1(0.4)	2(0.9)	1(0.6)	4(0.6)	21(1.1)	25(1)
Campylobacter jejuni	18(7.3)	22(9.7)	20(11.4)	60(9.3)	58(3.1)	118(4.7)
C. coli	1(0.4)	0	1(0.6)	2(0.3)	20(1.1)	22(0.9)
Shigellae	8(3.3)	21(9.3)	22(12.5)	51(7.9)	103(5.5)	154(6.1)
Salmonella	0	1(0.4)	1(0.6)	2(0.3)	21(1.1)	23(0.9)
EPEC	11(4.5)	8(3.5)	2(1.1)	21(3.2)	24(1.3)	45(1.8)
ETEC Group	9(3.7)	13(5.7)	5(2.8)	27(4.2)	87(4.6)	114(4.5)
EAEC	32(13.1)	28(12.3)	18(10.2)	78(12)	81(4.3)	159(6.3)
Virus						
Rotavirus	**115(46.9)**	**124(54.6)**	**39(22.2)**	**278(42.9)**	**124(6.6)**	**402(16)**
Adenovirus	24(9.8)	22(9.7)	8(4.5)	54(8.3)	44(2.4)	98(3.9)
Norovirus G1	3(1.2)	5(2.2)	3(1.7)	11(1.7)	21(1.1)	32(1.3)
Norovirus G2	10(4.1)	5(2.2)	2(1.1)	17(2.6)	25(1.3)	42(1.7)
Sapovirus	10(4.1)	4(1.8)	4(2.3)	18(2.8)	13(0.7)	31(1.2)
Astrovirus	5(2)	7(3.1)	5(2.8)	17(2.6)	38(2)	55(2.2)
Parasite						
Blastocystis hominis	0	0	0	0	11(0.6)	11(0.4)
Entamaeba histolytica	8(3.3)	13(5.7)	5(2.8)	26(4)	56(3)	82(3.3)
Giardia lamblia	25(10.2)	34(15)	33(18.8)	92(14.2)	189(10.1)	281(11.2)
Cryptosporidium spp.	37(15.1)	22(9.7)	12(6.8)	71(11)	87(4.6)	158(6.3)

New Frontiers in Diarrhoeal Diseases

Subclinical or Asymptomatic infections

The GEMS study showed that 72% of controls without diarrhoea also harbored one or more putative pathogens and 31% had two or more reflecting the fecally contaminated environment in which they live.

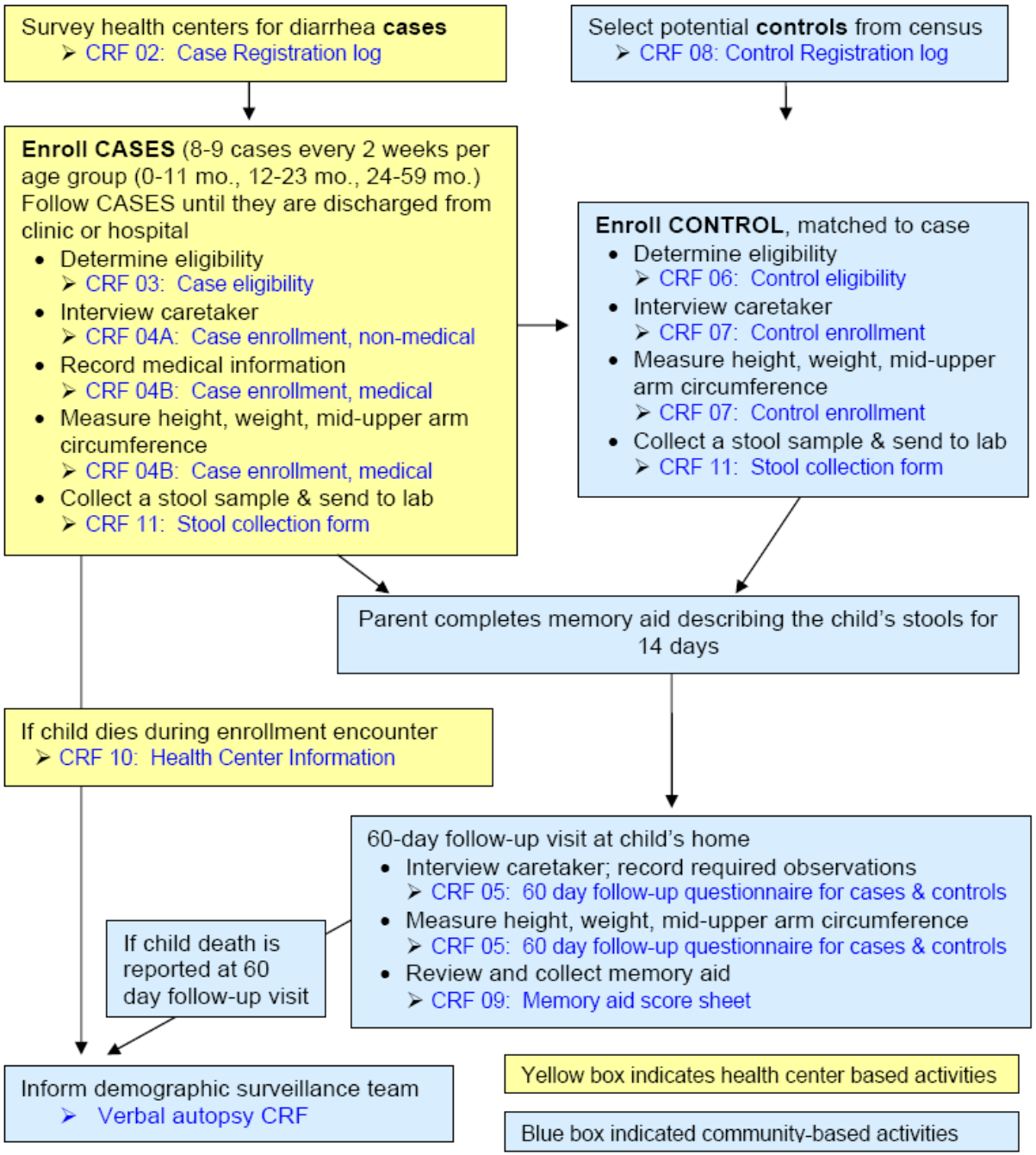
Case control study on diarrhoea in urban slums of Kolkata



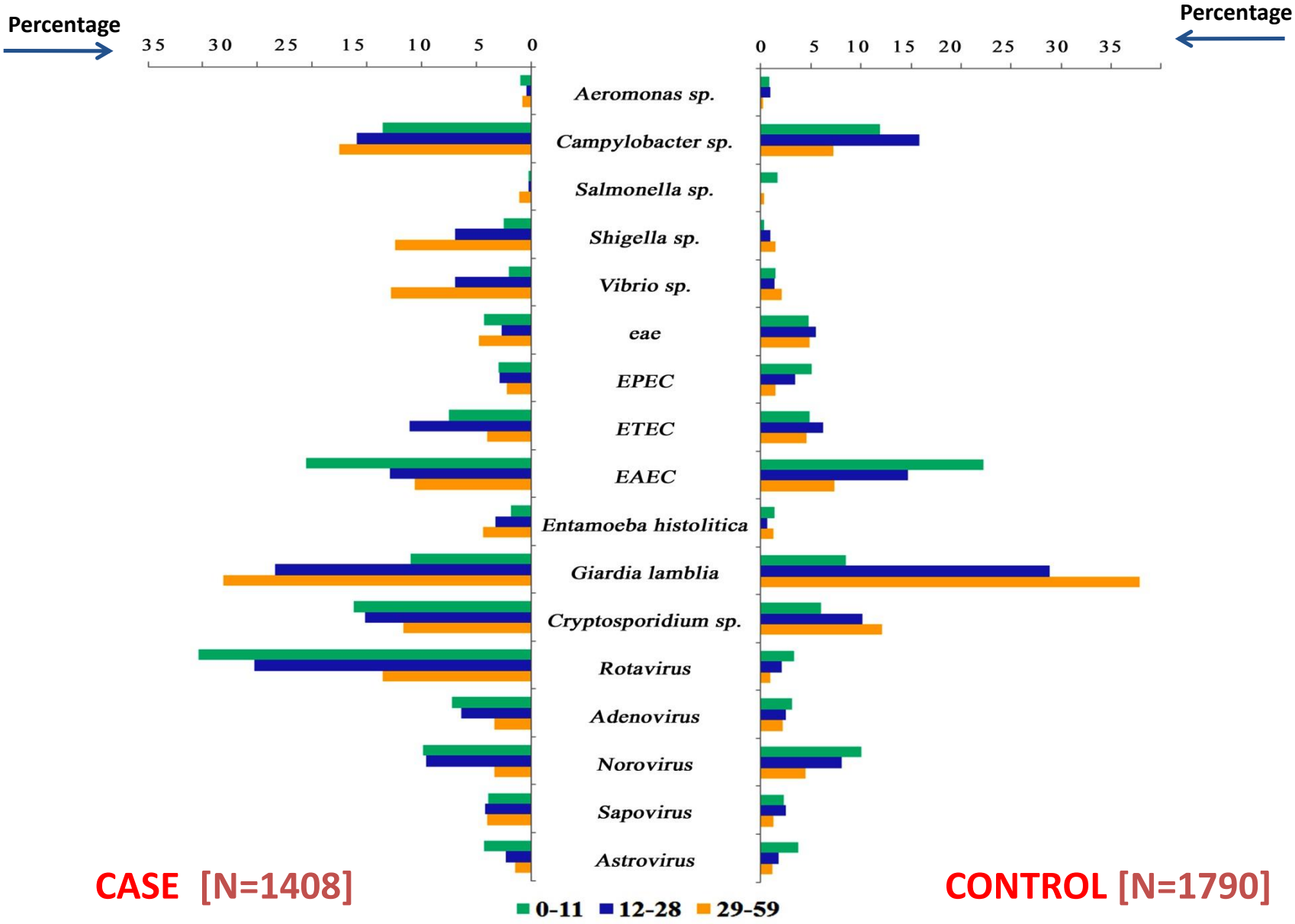
Principal Investigators
Dr. Dipika Sur
Dr. T. Ramamurthy
Global Enteric Multi Center Study

Study site: Map of Kolkata Municipal Corporation Area

STUDY OVERVIEW



Community Diarrhoea in an urban slum in Kolkata



CASE [N=1408]

CONTROL [N=1790]

Epidemiol. Infect. (2011), **139**, 919–926.
doi:10.1017/S0950268810001780

Role of probiotic in preventing acute diarrhoea in children: a community-based, randomized, double-blind placebo-controlled field trial in an urban slum

D. SUR[1]*, B. MANNA[1], S. K. NIYOGI[1], T. RAMAMURTHY[1], A. PALIT[1], K. NOMOTO[2], T. TAKAHASHI[2], T. SHIMA[2], H. TSUJI[2], T. KURAKAWA[2], Y. TAKEDA[3], G. B. NAIR[1] AND S. K. BHATTACHARYA[4]

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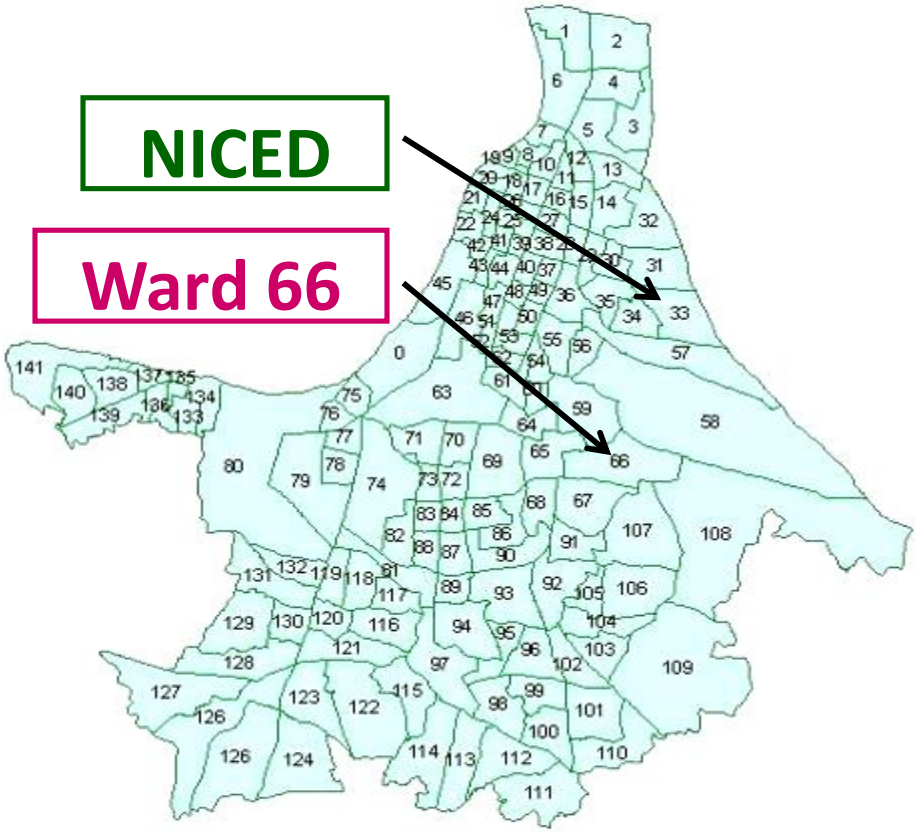
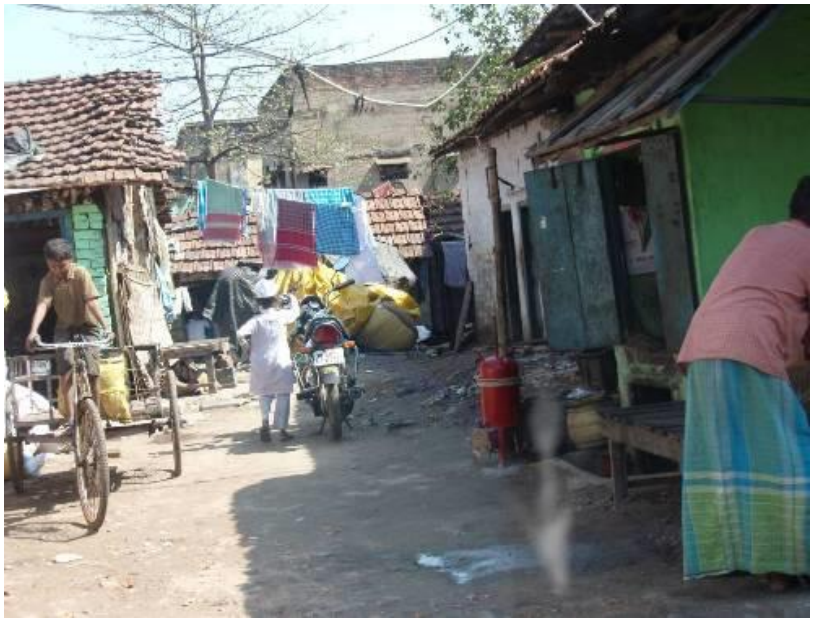
(*Accepted 29 June 2010; first published online 30 July 2010*)

NICED

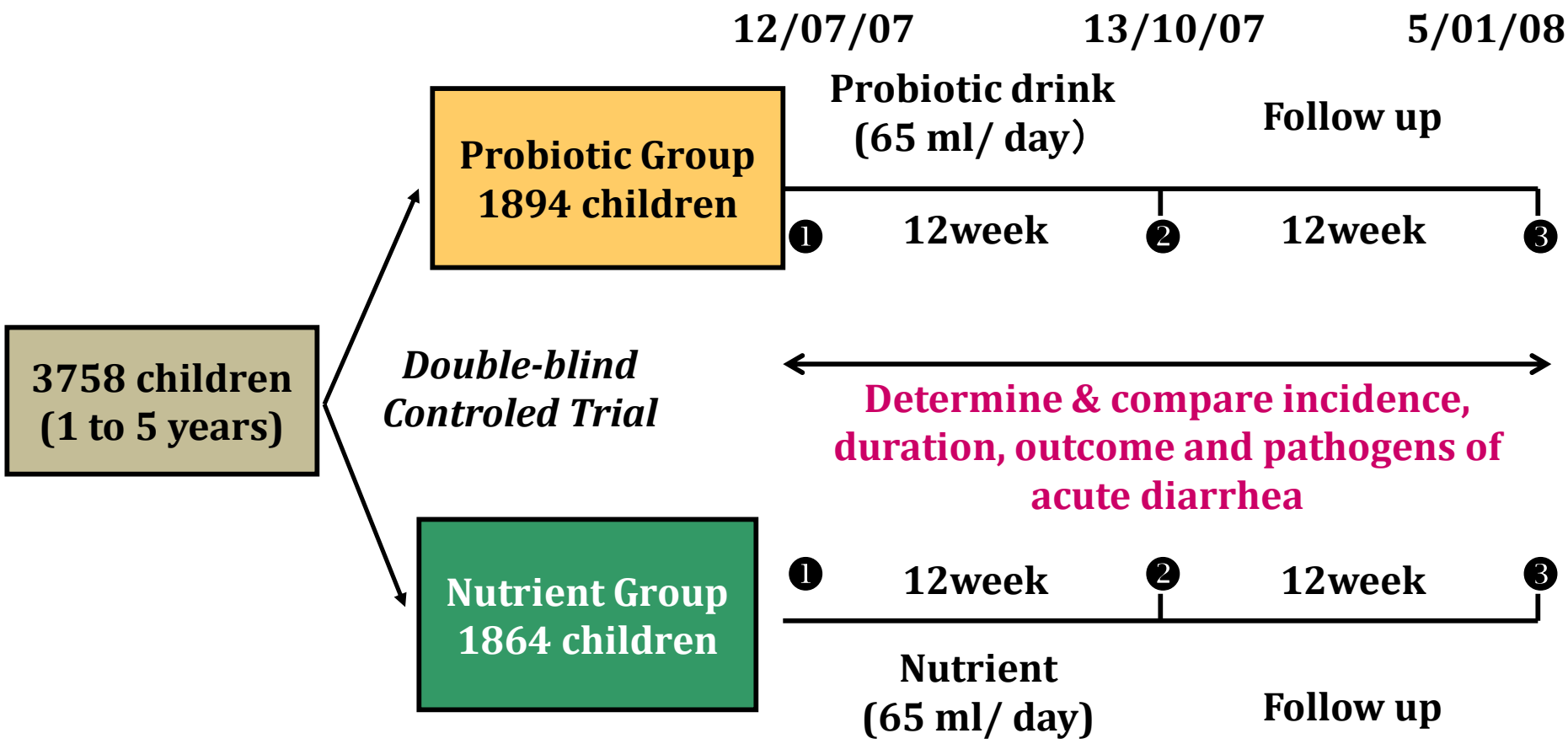
Ward 66



Kolkata Municipal Corporation

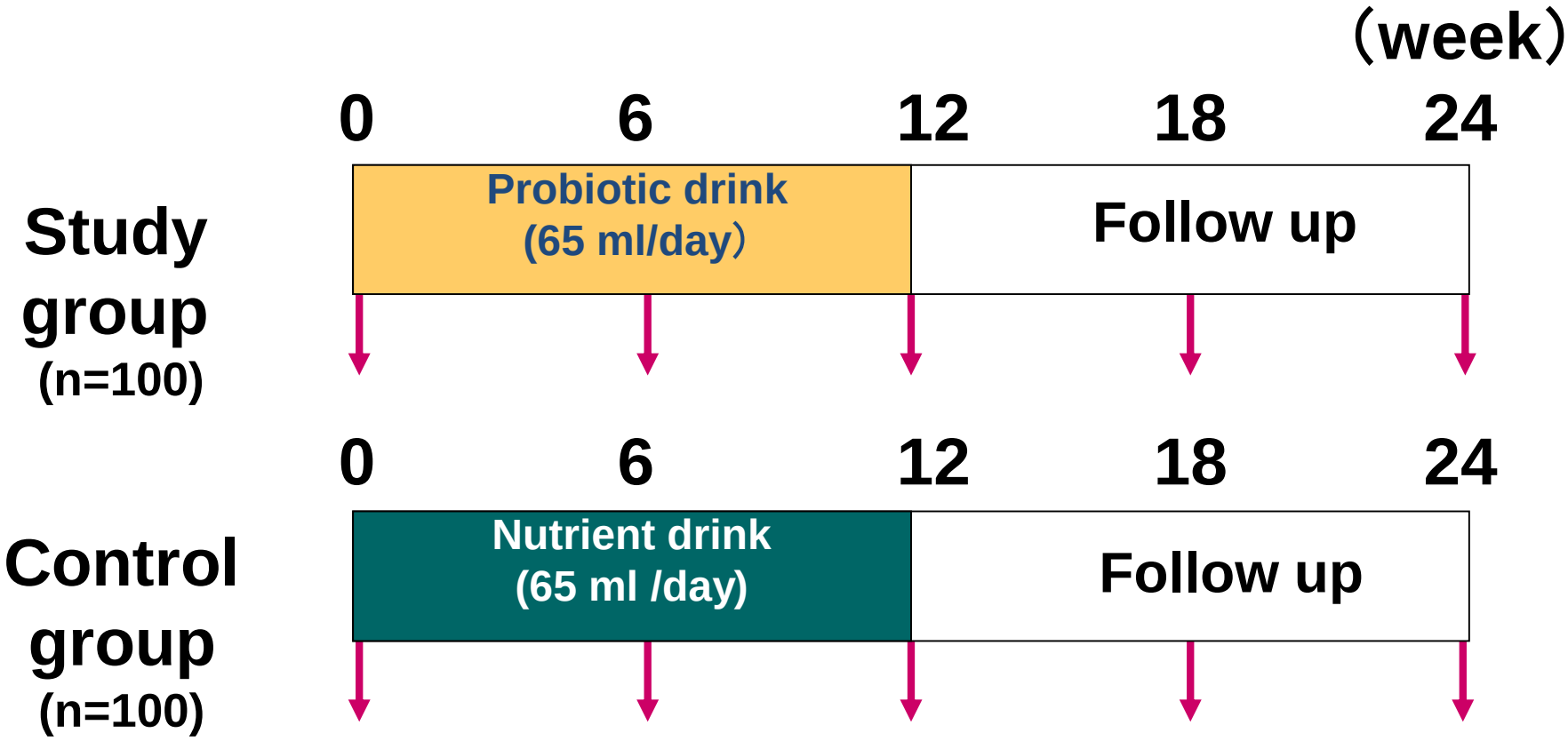


The plan of study



Nutritional assessment (height, weight and mid-arm circumference) were done at three points (❶,❷ and ❸, beginning, after 12weeks, and at the end of follow-up)

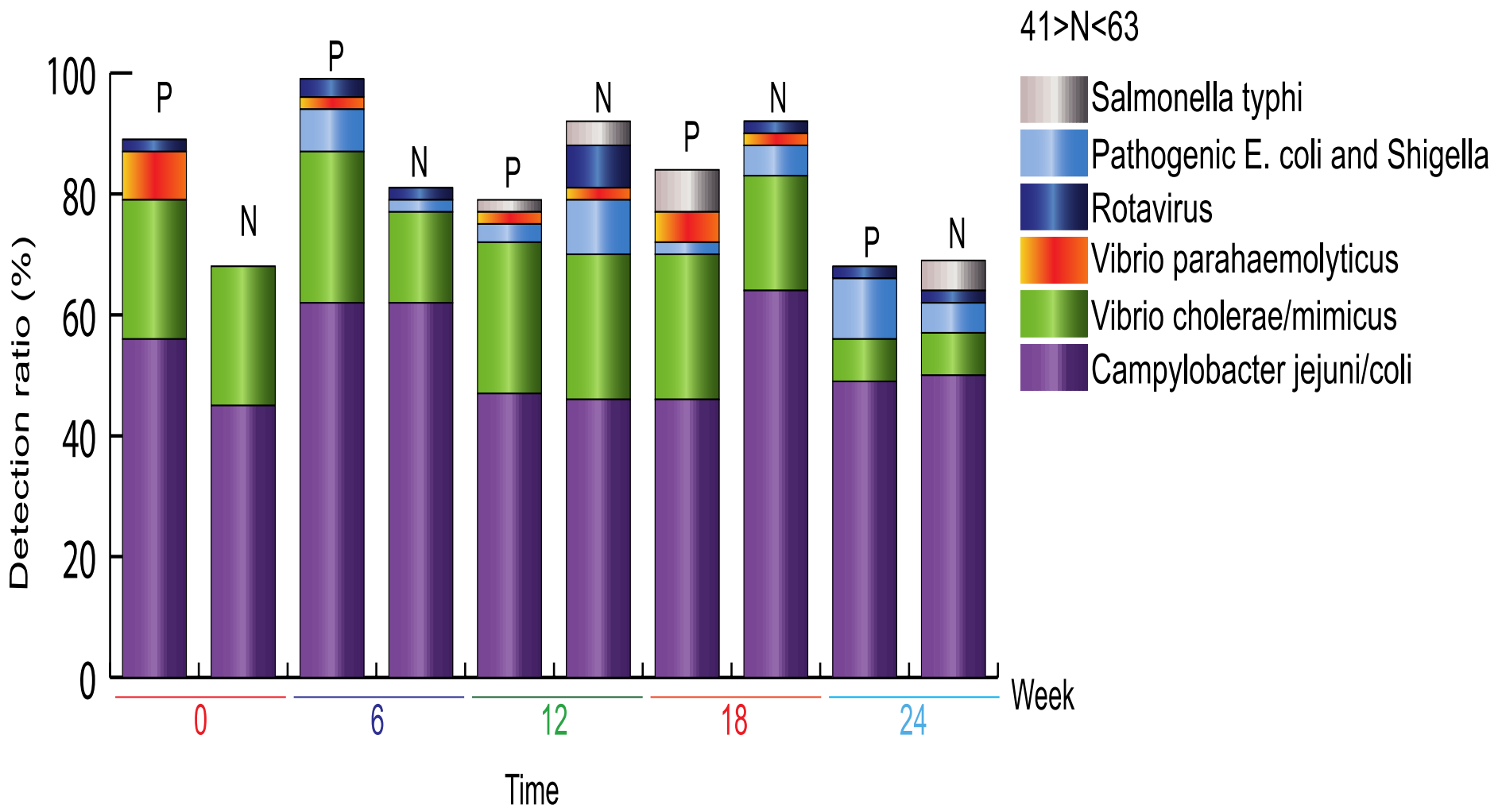
Periodical analysis of fecal microbiota



: collection of fecal sample for analysis of microbiota, pH and organic acid

Feces could be collected from 131 of the 200 subjects

Pathogens detected in the gut of apparently healthy children who participated in the probiotic trial



Detection of *Vibrio cholerae/mimicus* in faeces collected from healthy children

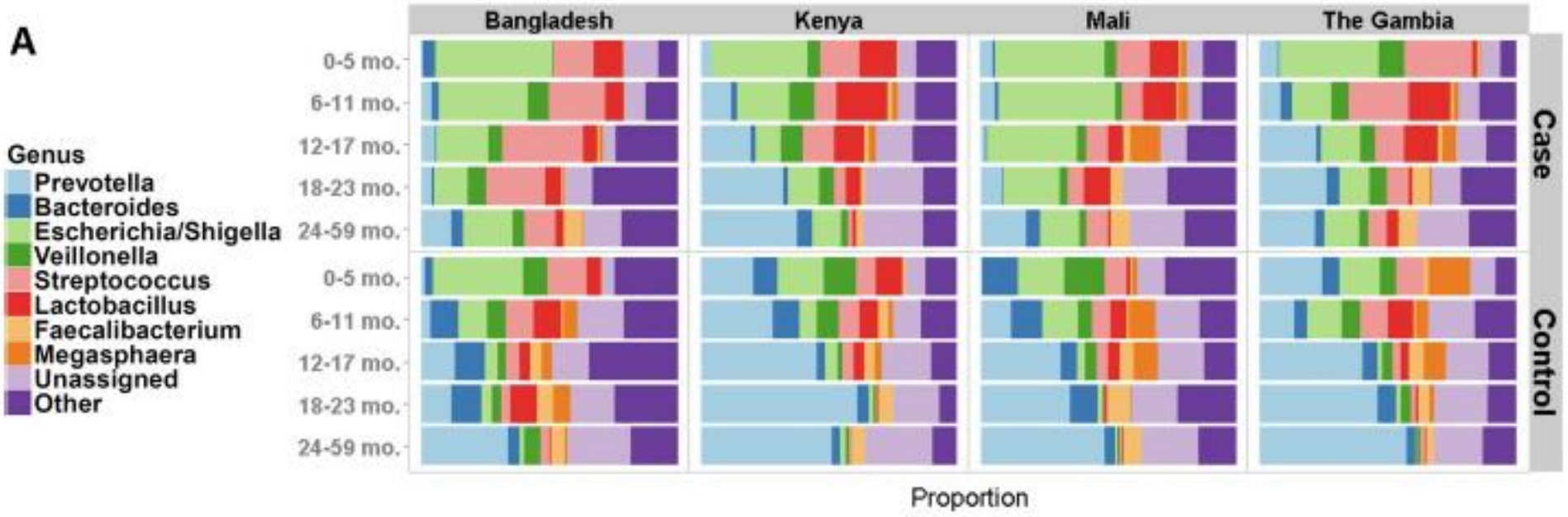
Detection frequency	Detection rate	(%)	Bacterial counts
1	42/133[a]	31.6	4.2 ± 1.5[b]
2	21/133	15.8	3.8 ± 1.0
3	6/133	4.5	4.5 ± 1.3
4	0/133	0.0	-
5	1/133	0.8	4.3 ± 0.9
Total	70/133	52.6	4.1 ± 1.3
Twice or more	28/133	21.1	4.0 ± 1.1
Twice or more in a row	17/133	12.8	4.2 ± 1.1

[a] No. of positive subjects/No. of subjects tested

[b] Mean ±SD, log $_{10}$ cells/g feces

Comparison of diarrheal and non-diarrheal stool

Proportional abundance of genera in non-diarrheal controls and MSD cases in different age categories



Each color represent a different group. The order and color for each group is the same for controls and MSD cases

Mihai Pop et al., Genome Biology 2014,15:R76

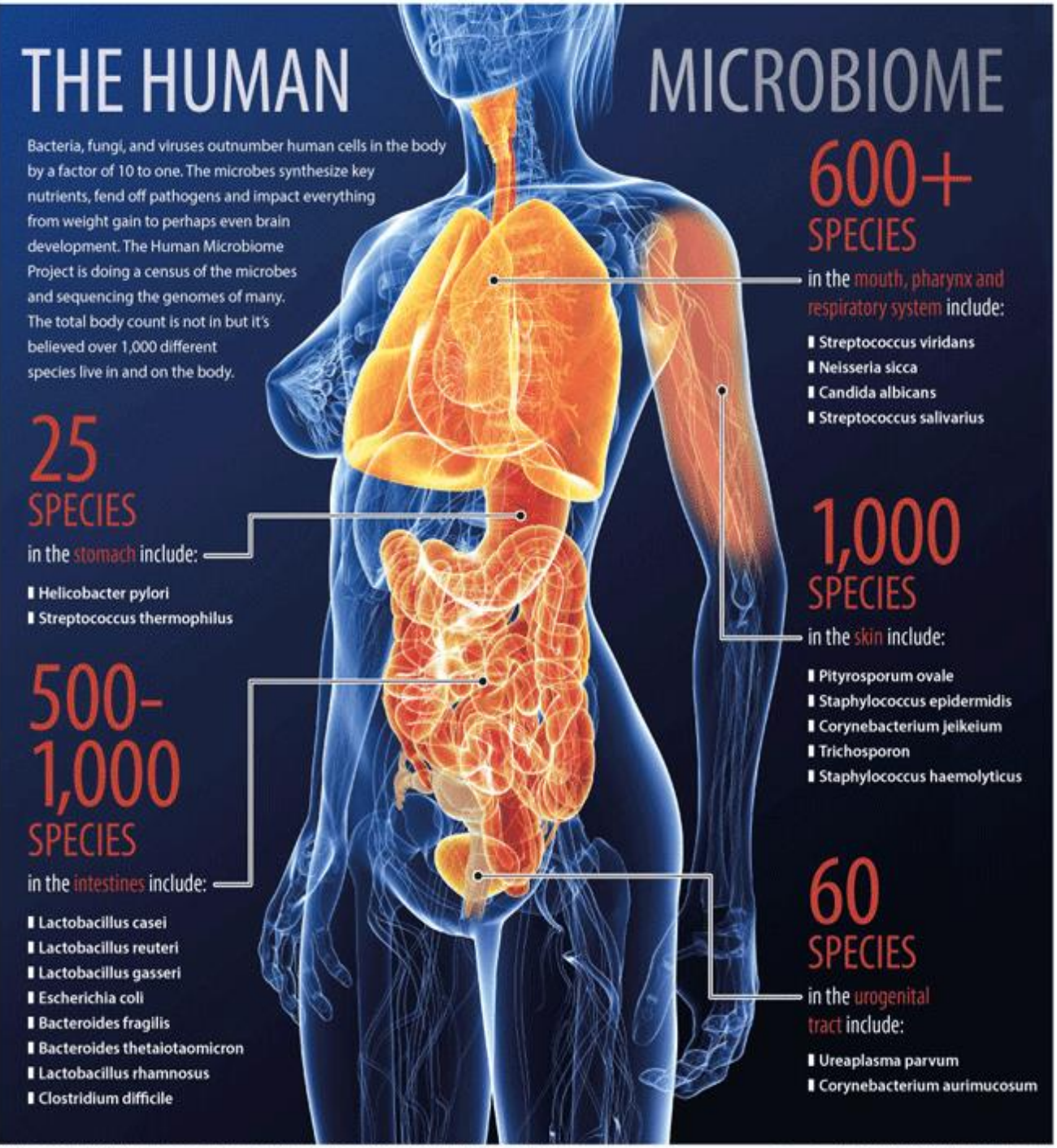
Subclinical (asymptomatic) infections Environmental Enteric Dysfunction

- Mounting and diverse evidence suggests that subclinical infections with diarrhoea pathogens can cause physiological and structural alterations of the gut with diverse consequences on child nutrition and growth.
- Subclinical infections reduce nutrient absorption and impair growth by many of the same mechanisms present during clinical episodes.
- *Giardia intestinalis* causes diarrhoea with growth retardation in infants; often identified in the stools of asymptomatic children in endemic areas.

Because asymptomatic infections were twice as common as diarrhoea their ultimate effects might exceed those of clinical diarrhoea

New Frontiers in Diarrhoeal Diseases

Diarrhoeal Diseases and Intestinal microbiota



Human Microbiome Project

A total of 4,788 specimens from 242 screened and phenotyped adults (129 males, 113 females) were used for this study, from a cohort of 300 individuals

Women were sampled at 18 body habitats, men at 15. 2

Source: NIH

Gut Microbiota

- Bacteroidetes and Firmicutes make up around 90% of the gut microbiota
- Each individual harbors his/her own distinctive pattern of gut microbial communities
- For a given individual, the fecal microbiota remains remarkably stable over a person's lifetime

Gut Microbiota - Functions

- Prevents colonization by pathoge
- “Educates the immune system”
- Metabolic role
  - Caloric salvage
  - Produces
- SCFA
- Vitamin K and folate
- Participates in drug metabolism
- Activates 5-ASA
- Deconjugates bile acids

LETTER

doi:10.1038/nature13738

Members of the human gut microbiota involved in recovery from *Vibrio cholerae* infection

Ansel Hsiao[1], A. M. Shamsir Ahmed[2,3], Sathish Subramanian[1], Nicholas W. Griffin[1], Lisa L. Drewry[1], William A. Petri Jr[4,5,6], Rashidul Haque[3], Tahmeed Ahmed[3] & Jeffrey I. Gordon[1]

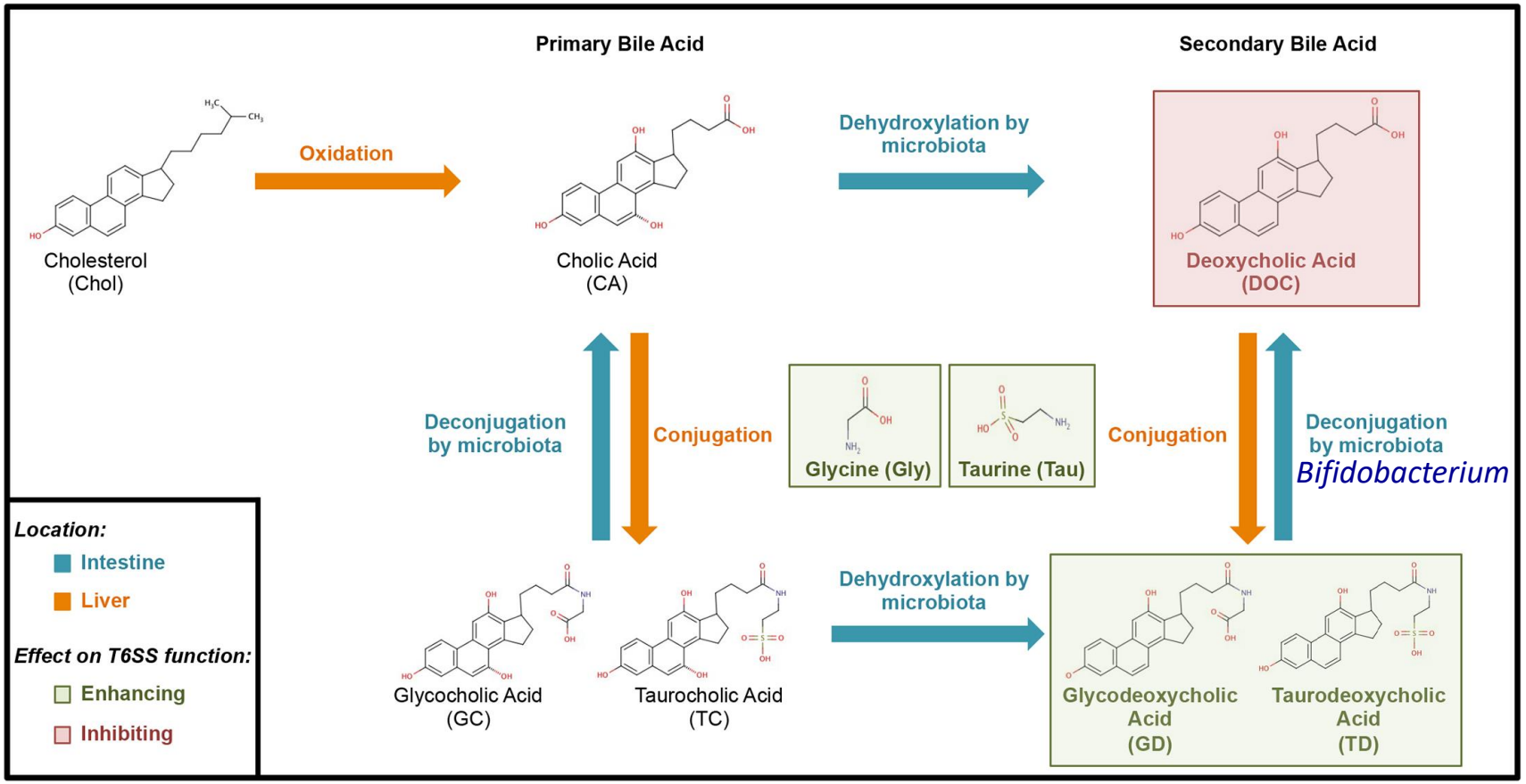
Given the global burden of diarrhoeal diseases[1], it is important to understand how members of the gut microbiota affect the risk for, course of, and recovery from disease in children and adults. The acute, voluminous diarrhoea caused by *Vibrio cholerae* represents a dramatic example of enteropathogen invasion and gut microbial community disruption. Here we conduct a detailed time-series metagenomic study of faecal microbiota collected during the acute diarrhoeal and recovery phases of cholera in a cohort of Bangladeshi adults living in an area with a high burden of disease[2]. We find that recovery is characterized by a pattern of accumulation of bacterial taxa that shows similarities to the pattern of assembly/maturation of the gut microbiota in healthy Bangladeshi children[3]. To define the underlying mechanisms, we introduce into gnotobiotic mice an artificial community composed of human gut bacterial species that directly correlate with recovery from cholera in adults and are indicative of normal microbiota maturation in healthy Bangladeshi children[3]. One of the species, *Ruminococcus obeum*, exhibits consistent increases in its relative abundance upon *V. cholerae* infection of the mice. Follow-up analyses,

to D-Ph4. Every diarrhoeal stool was collected from every participant. Faecal samples were also collected every day for the first week after discharge (recovery phase 1, R-Ph1), weekly during the next 3 weeks (R-Ph2), and monthly for the next 2 months (R-Ph3). For each individual, we selected a subset of samples from D-Ph1 to D-Ph3 (Methods), plus all samples from D-Ph4 to R-Ph3, for analysis of bacterial composition by sequencing PCR amplicons generated from variable region 4 (V4) of the 16S ribosomal RNA (rRNA) gene (Supplementary Information, Extended Data Fig. 1a and Supplementary Table 3). Reads sharing 97% nucleotide sequence identity were grouped into operational taxonomic units (97%-identity OTUs; Methods).

We identified a total of 1,733 97%-identity OTUs assigned to 343 different species after filtering and rarefaction (Methods). *V. cholerae* dominated the microbiota of the seven patients with cholera during D-Ph1 (mean maximum relative abundance 55.6%), declining markedly within hours after initiation of oral rehydration therapy. The microbiota then became dominated by either an unidentified *Streptococcus* species (maximum relative abundance 56.2–98.6%) or by *Fusobacterium* species (19.4–

Hsiao et al., 2014, Nature

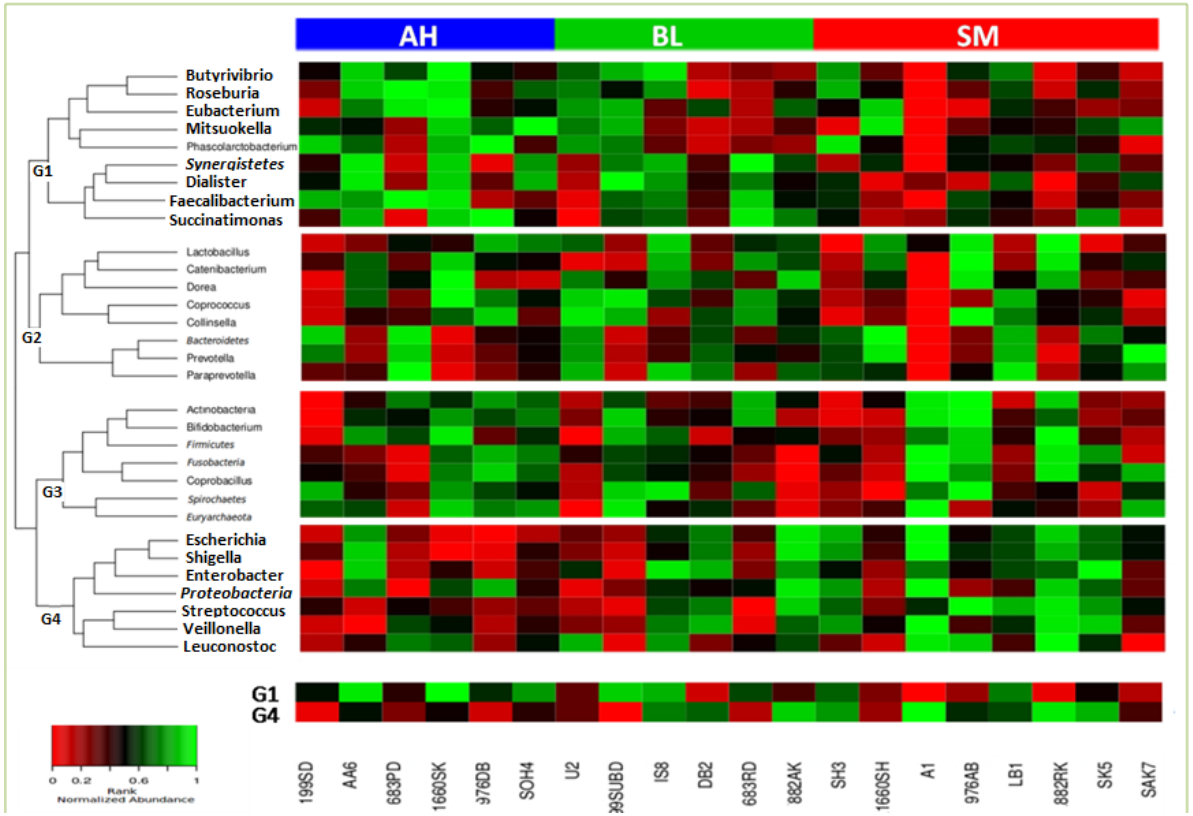
Bifidobacterium can repress *V. cholerae* virulence by modifying bile acids

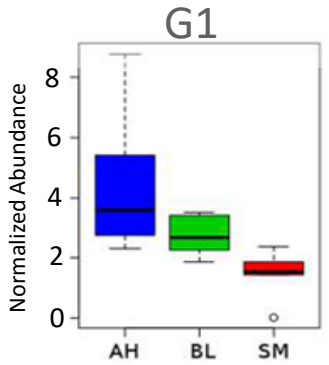


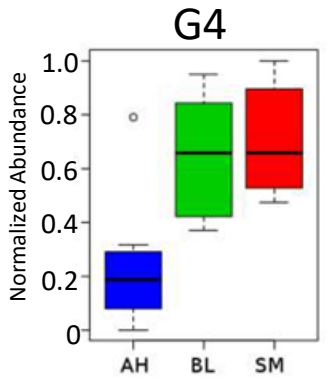
Microbiota modify bile acids to inhibit T6SS-mediated killing of commensal bacteria. This interplay is novel interaction between commensal bacteria, host factors, and Vc

Bachmann et al., 2015, PNTD

Variation of Microbiota across nutritional status







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PLOS ONE 2014

Gut Microbiomes of Indian Children of Varying Nutritional Status

Tarini Shankar Ghosh[1☯], Sourav Sen Gupta[2☯], Tanudeep Bhattacharya[1], Deepak Yadav[1], Anamitra Barik[3], Abhijit Chowdhury[3,4], Bhabatosh Das[2], Sharmila S. Mande[1*], G. Balakrish Nair[2*]

Our Research Questions

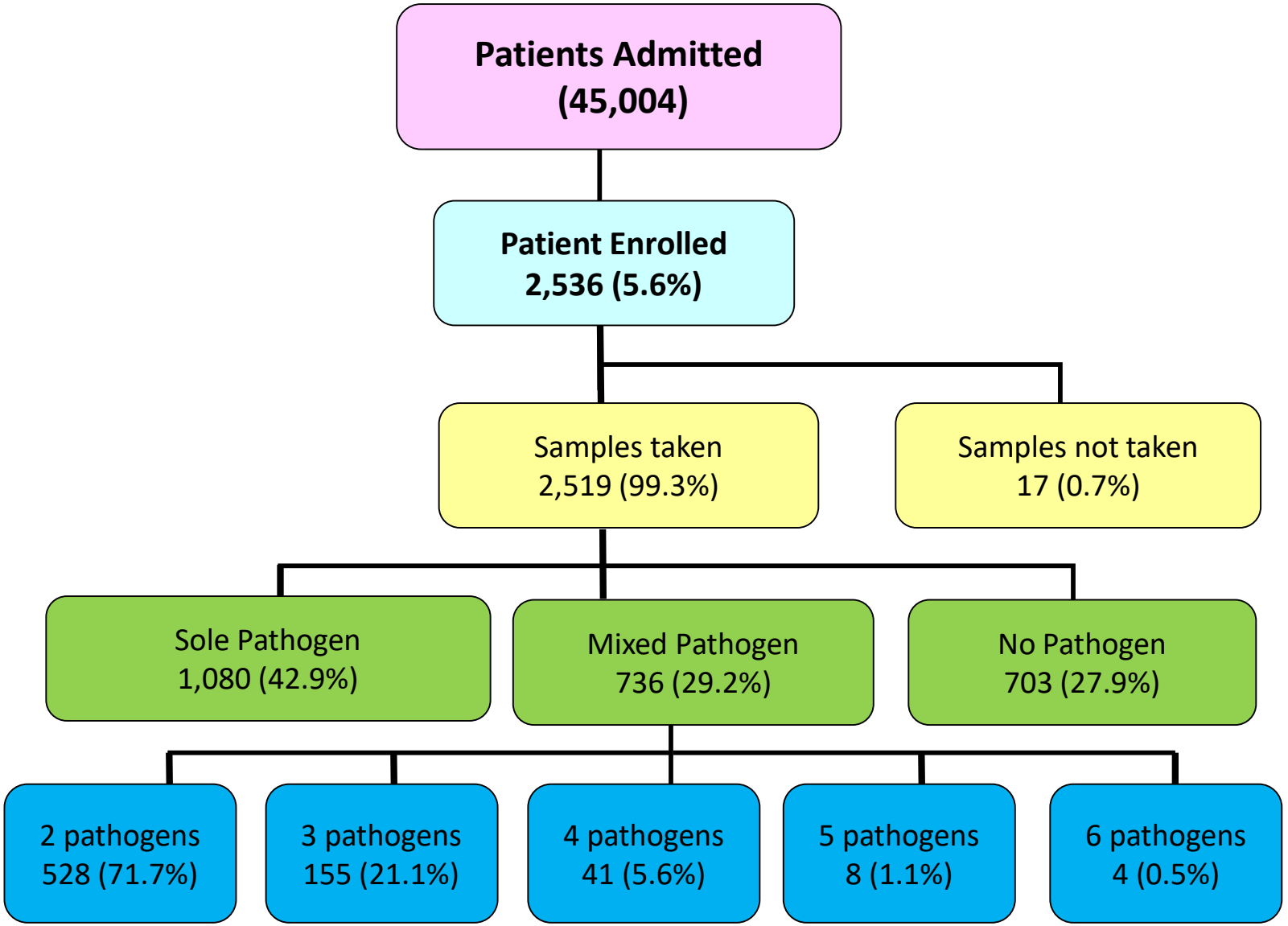
- What are pathogens doing in the gut of apparently healthy children and adults?
- Why are they present in low numbers?
- How does commensals restrain pathogen growth
- Why are the pathogens not eliminated from the gut microbiota?
- Does the presence of pathogens induce inflammation and
- What is the role of pathogens?

New Frontiers in Diarrhoeal Diseases

Polymicrobial Infections

Diarrhoea at the Infectious Diseases Hospital, Kolkata

November 2007 and October 2009



Diarrheagenic Pathogens in Polymicrobial Infections

Brianna Lindsay,[1] T. Ramamurthy,[1] Sourav Sen Gupta, Yoshifumi Takeda, Krishnan Rajendran, G. Balakrish Nair, and O. Colin Stine

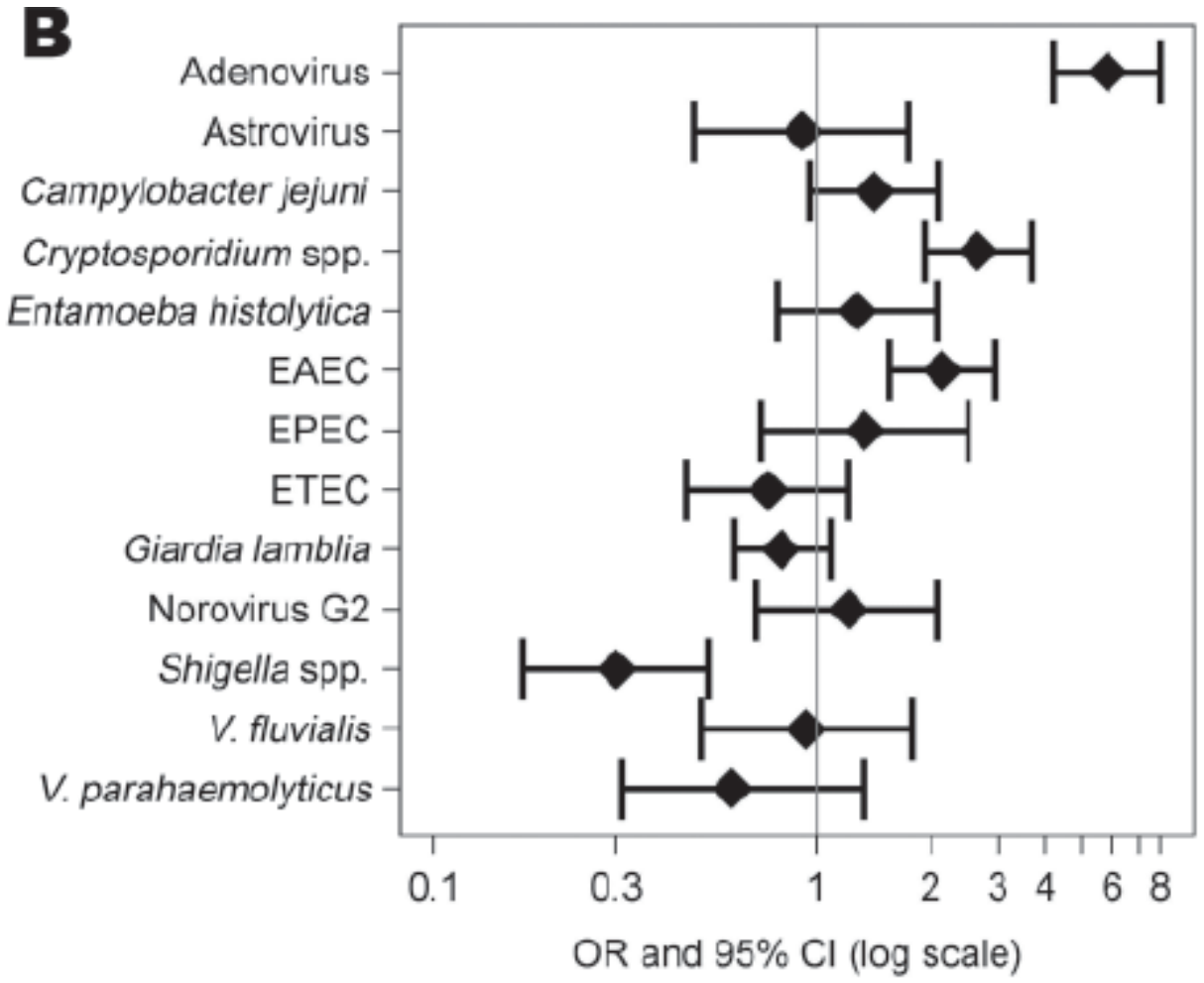
Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 17, No. 4, April 2011

What is the relationship between pathogens associated with mixed infections?

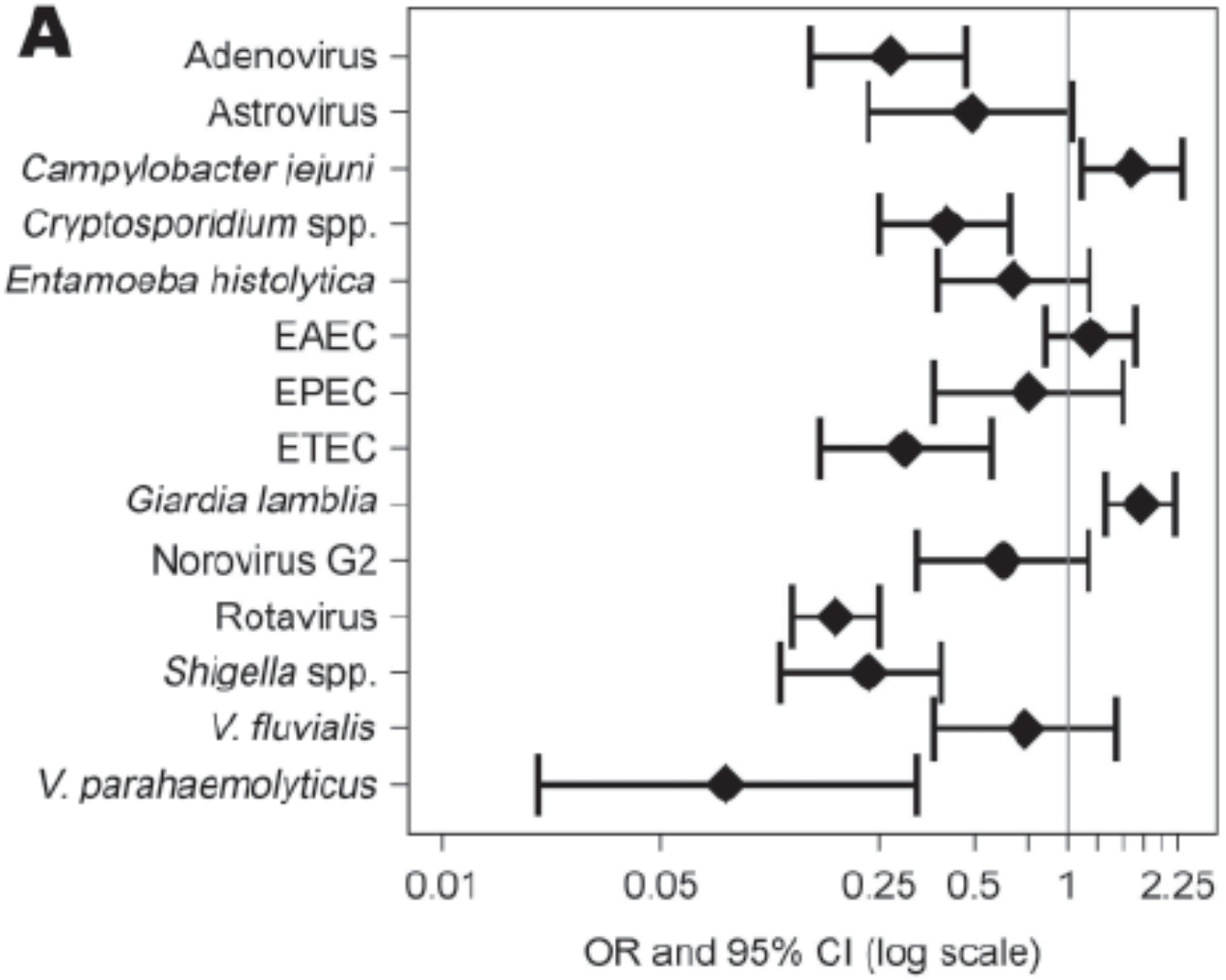
Testing Possible Associations

To test for possible associations, we used the Fisher Exact test to compare pairs of pathogens (1, both or neither) with an independent assortment based on the overall frequency with which pathogens were detected. To establish criteria for statistical significance, we calculated p values, odds ratios and 95% confidence intervals.

Odds ratios (ORs) showing odds of rotavirus co-occurring with various other pathogens



Odds ratios (ORs) showing odds of *Vibrio cholerae* co-occurring with various other pathogens



Polymicrobial infections associated with *Vibrio cholerae* and Rotavirus cases was non random

Interventions for Diarrheal Diseases

Category	Options
Therapeutic	• Oral rehydration solutions • Antimicrobials for bloody diarrhea or dysentery • Nutritional treatment of persistent diarrhea • Zinc supplementation
Preventive	• Protected safe water • Handwashing sanitary disposal of fecal waste • Vaccines • Improved nutrition, vitamin A and Zinc.

Vaccines against diarrheal diseases

- Rotavirus – Two vaccines – Merck and GSK are widely used
- A less expensive Indian manufactured vaccine named Rotavac

  has been pre qualified by WHO and is approved for use in India
- Cholera vaccine – Dukoral and Shancol

Summary

- The burden of DD in children under age five years in LIMCs reduced dramatically
- Although there are no magic bullets to control the incidence of DD the following are highly effective:
  - Improved nutrition of young children
  - Water and sanitation improvement
  - Hand washing and implementation of simple but highly effective interventions such as ORS
  - Appropriate use of antibiotics
  - The role and cause of EED

Thank you for your attention