Yale University, CT Emerging Infections Program

Sentinel Surveillance for Unexplained Diarrhea

Primary goal: To determine the burden and etiology of unexplained diarrhea among a group of patients in New Haven, Connecticut.

Objectives

1) Establish surveillance for diarrheal illness among patients who receive care at the YNHH Primary Care Centers (PCC) and Emergency Department (ED);

2) Collect epidemiological data and stool specimens from patients with diarrheal illness and healthy controls;

3) Perform comprehensive laboratory analysis for bacterial, viral and parasitic pathogens on all stool samples collected from case patients and controls;

4) Develop a bank of stored specimens for future testing for new or emerging enteric pathogens.

Eligibility Criteria

Case - Any patient who presented to the pediatric or adult PCC or ED with self described diarrheal illness.

Control - Any patient who presented to the pediatric or adult PCC for a well visit or to the ED for a non-life threatening illness or injury who had not had diarrheal illness in the past 30 days.

Those not providing a stool specimen were excluded.

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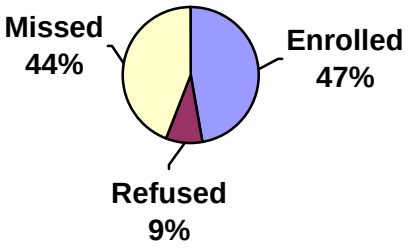
Subject Recruitment

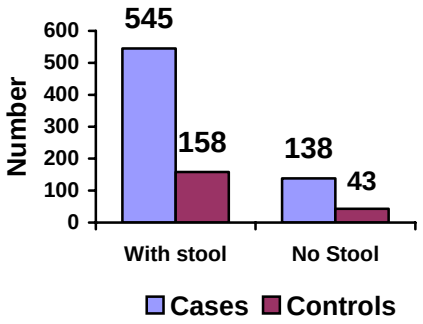
May 1, 2002—September 14, 2004

Eligible Cases N=1,445



A total of 1,445 eligible case patients were identified during the study period. Of these, 683 (47%) were enrolled, 637 (44%) were missed (surveillance officer not on duty, not notified, or asked not to approach patient), and 125 (9%) refused to participate.

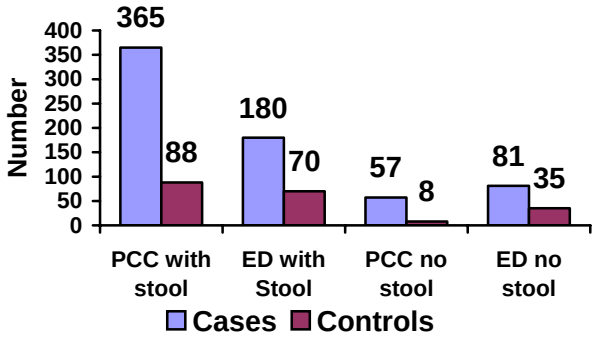
Case and Control Participation



Of the 683 enrolled case patients, 545 (80%) provided a stool specimen and 138 (20%) did not provide a stool specimen and were excluded.

Of the 201 enrolled controls, 158 (79%) provided a stool specimen and 43 (21%) did not provide a stool specimen and were excluded.

Enrollment by Hospital Site



Of the 545 case patients with stool, 365 (67%) were recruited from the Primary Care Centers and 180 (33%) were recruited from the Emergency Department.

Of the 158 control subjects with stool, 88 (56%) were recruited from the Primary Care Centers and 70 (40%) were recruited from the Emergency Department.

Participant Demographics

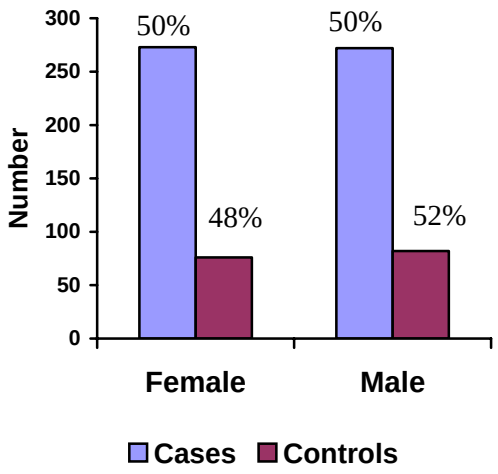
Race, Ethnicity and Sex by Case/Control Status

Race by Case/control status

Race	Cases	Controls
White	205 (38%)	45 (29%)
Black	186 (34%)	87 (55%)
Asian/Pacific Islander	10 (2%)	3 (2%)
American Indian/ Alaska Native	3 (<1%)	2 (1%)
Other/Unknown	141 (26%)	21(13%)
Ethnicity		
Hispanic	196 (36%)	31 (20%)
Non-Hispanic	345 (63%)	126 (80%)
Unknown	2 (<1%)	1 (<1%)

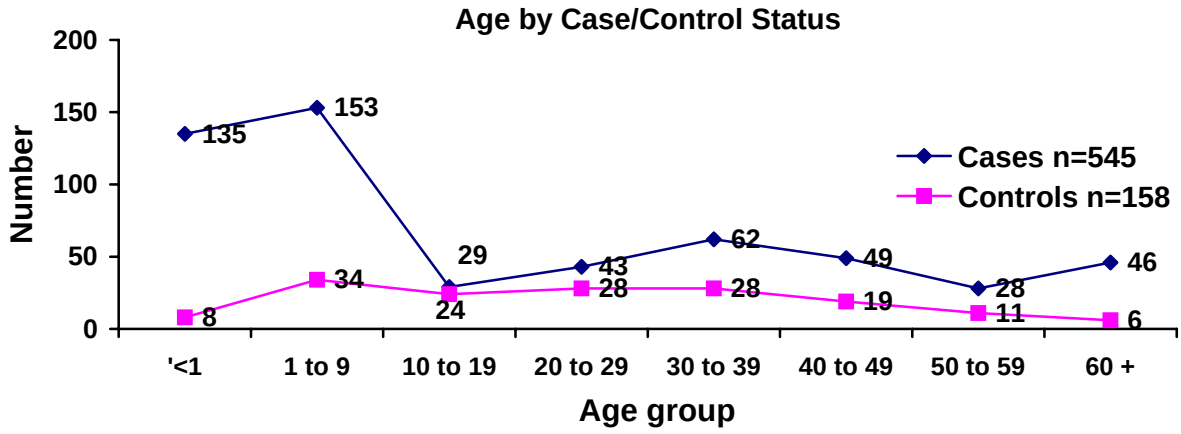
Included cases were more likely to report white (38% vs. 29%) race or other/unknown race (26% vs 13%) than were controls. Almost twice as many cases reported Hispanic ethnicity (36%) than did controls

Sex by Case/control status



There was no difference in sex between included case patients and included control subjects.

Age Group by Case/Control Status



Of 545 included case patients, 57% were 18 years of age or younger. Of the 158 included control subjects, 36% were 18 years of age or younger.

Laboratory Testing Overview - Distribution of Bacteria, Parasites and Viruses

Laboratory testing of stool specimens from cases and controls included the following:

Bacterial testing: *Salmonella spp., Shigella spp., Yersinia, Aeromonas, Plesiomonas, Vibrio, Campylobacter jejuni* and other presumptive *Campylobacter* spp., *E. coli* O157 and non-O157 STECs, *Listeria monocytogenes, Clostridium difficile* and *C. perfringens* toxin.

Parasitic testing: Routine examination for protozoa and helminthes, *Cryptosporidium parvum, Cyclospora, Isospora,* and *Microsporidia*.

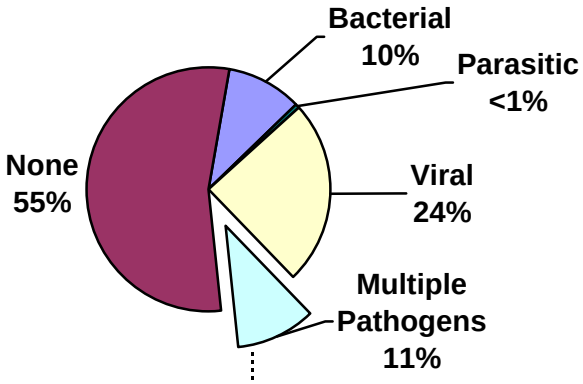
Viral testing: adenovirus, rotavirus, astrovirus, norovirus, and sapovirus.

Positive Tests by Site and Organism

PCC = Primary Care Center
ED = Emergency Department

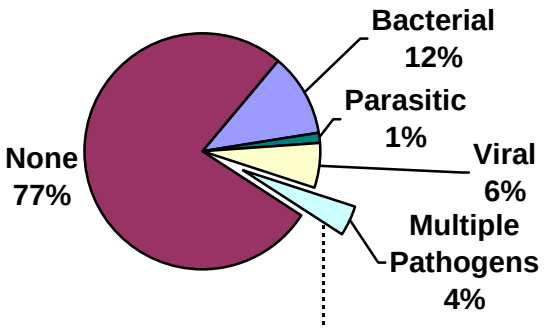
Hospital Site	Bacteria	Virus	Parasite	Total
PCC	**94**	**143**	**7**	**244**
Adult	24	22	5	51
Pedi	70	121	2	193
ED	**35**	**55**	**2**	**92**
Adult	26	33	1	60
Pedi	9	22	1	32
Total	**129**	**198**	**9**	**336**

Percentage of pathogens identified from case-patient stool specimens n=545



Category of multiple pathogens identified	Cases
2 or more bacteria	5
2 or more viruses	14
Bacteria and parasite	3
Bacteria and virus	35
Virus and parasite	1

Percentage of pathogens identified from control-subject stool specimens n=158



Category of multiple pathogens identified	Controls
2 or more viruses	1
Bacteria and parasite	6
Bacteria and virus	1

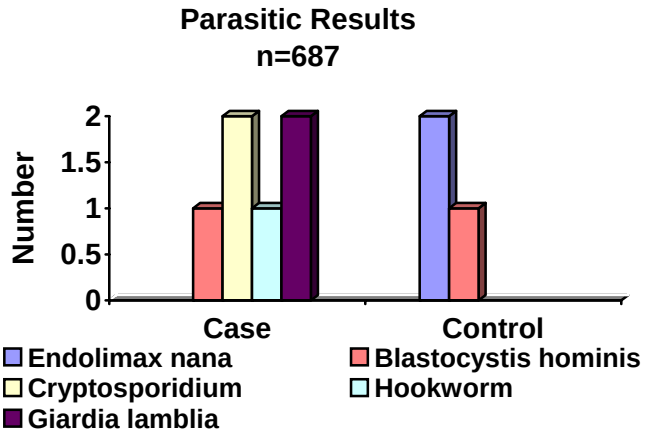
Microbial Testing Results

Bacterial culture, wet-mount examination and special staining methods, enzyme immuno-assay (EIA) and polymerase chain reaction (PCR) were performed on stool specimens from both case patients and control subjects to identify possible bacterial and/or parasitic pathogens.

Bacterial Results by Case/Control Status

Bacteria	Case/ No. tested	Control/ No. tested
Salmonella	14/544 (3%)	2/157(1%)
Shigella	2/544 (<1%)	0
Yersinia	4/544(<1%)	2/157(1%)
Aeromonas	4/544(<1%)	2/157(1%)
Campylobacter jejuni	9/544(2%)	0
Campylobacter concisus	24/481(5%)	4/157(3%)
Sutterella wadsworthensis	27/481(6%)	13/157(8%)
MRSA	1/544 (<1%)	0
Clostridium difficile toxin	11/516(2%)	1/156(1%)
Clostridium perfringens toxin	8/518(2%)	1/156(1%)
Total Positive Tests	104	25

* Pathogenicity of *Campylobacter concisus* and *Sutterella wadsworthensis* is not known.



There were 138 bacterial and parasitic organisms identified among 101 case patients and 28 control subjects. Of the 138 organisms, 129 (93%) were bacterial, and 9 (7%) were parasitic. The most common bacterial isolate identified among both case patients and control subjects was *Sutterella (*6% and 8%). Of the 544 case patients, 19% were positive for a bacteria or parasite. Of the 157 control subjects, 16% were positive for a bacteria or parasite.

There were 9 patients tested that had more than one bacterial and/or parasitic pathogen identified. There were 11 different *Salmonella* serotypes and 5 parasitic organisms. There were no positive specimens for EHEC by EIA or *E. coli* O157 isolated by culture.

Viral PCR Results by Case/Conrol Status

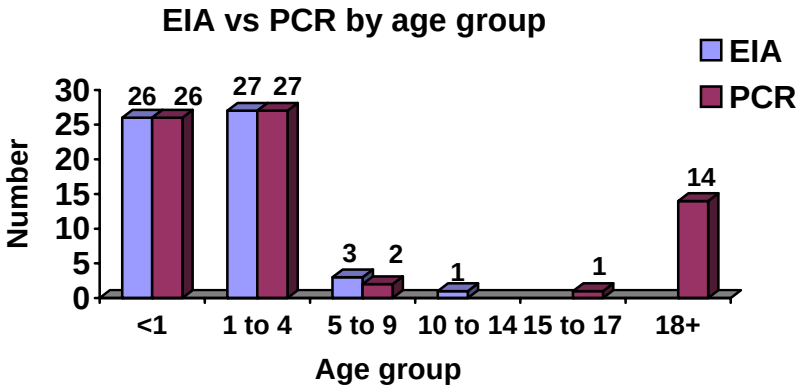
	Cases (No.=522)	Controls (No. =156)
Adenovirus	23 (4%)	0
Astrovirus	19 (4%)	3 (2%)
Rotavirus	69 (13%)	2 (1%)
Norovirus	81 (16%)	12 (8%)
Sapovirus	3 (1%)	1 (1%)
Total	195 (37%)	18 (12%)

Polymerase Chain Reaction (PCR) testing was performed on 678 specimens; 522 from case patients and 156 from control subjects. There were 23 specimens from cases and 2 specimens from controls not tested due to insufficient sample. DNA was extracted from whole stool and amplified using primer sets targeting the genome of *adenovirus*, *astrovirus*, *rotavirus*, *norovirus*, and *sapovirus*.

Viral DNA or RNA was amplified from the stool of 213 participants; 195 from case patients and 18 from control patients. Fourteen case patients and one control patient were positive for two or more viral pathogens.

CLINICAL RESEARCH LABORATORY SPECIAL PROJECTS

COMPARISON OF EIA AND PCR METHODS FOR *ROTAVIRUS* BY AGE GROUP



All stools were tested for rotavirus by PCR. Stools from pediatric subjects (<18 years of age) were also tested for rotavirus by EIA. There were 61 pediatric specimens that were positive for *rotavirus* by either PCR or EIA. Of these, 52 (85%) were positive by both methods; 4 (7%) specimens were positive by PCR only, and 5 (8%) were positive by EIA only. PCR testing of adult stool specimens yielded 14 specimens positive for *rotavirus*.

ANTIMICROBIAL RESISTANCE TESTING ON NORMAL FLORA

Antibiotic susceptibility of select isolates of normal stool flora was determined to investigate the reservoir of antibiotic resistant organisms in stool. Stool was enriched for *Enterococcus* and plated on media containing gentamicin and on media containing synercid. Stool was enriched for *E. coli* and plated on media containing nalidixic acid and on media containing ceftazidime. Stool was also directly plated onto MacConkey agar with ceftazidime. Speciation and resistance confirmation by broth microdilution (Sensititre©) or disk diffusion was completed for a subset of isolates using NCCLS breakpoints.

Organisms considered a normal component of fecal flora were resistant to clinically important antibiotic drugs. Resistant organisms among normal fecal flora may become opportunistic pathogens and may carry a pool of transferable resistance elements.

Antibiotic (organism)	Total isolates	Non-susceptible isolates
High-level Gentamicin (*Enterococcus spp*).	551	24 (4%)
Synercid (*Enterococcus faecium*)	558	22 (4%)
Nalidixic Acid (*E.coli*)	523	23 (4%)
Ceftazidime (*E.coli*)	537	6 (1%)
Ceftazidime (*Enterobacteriaceae)	539	16 (3%)
Ceftazidime (non-*Enterobacteriaceae)	539	8 (2%)

E. COLI VIRULENCE GENES

PCR testing for diarrheagenic *E.coli* virulence genes was completed on mixed *E. coli* isolates from 497 case patients and 151 control patients. There were 54 isolates positive for at least one virulence gene. The most common were Eagg and eae. Shiga toxin (ST1) PCR resulted in one hlyA being identified.

V Gene	Cases	Controls	Total specimens	Total positives
ipah	3/497	0/151	648	3
Eagg	20/497	2/151	648	22
eae	26/497	3/151	648	29
Eaf	0/26	0/3	29	0
Bfp	2/26	0/3	29	2
LT1	2/497	1/151	648	3
ST1	1/497	0/151	648	1
Hlya	1/497	0/151	648	1

Thank You for your support!

The EIP and SSUD study personnel would like to extend our sincere gratitude to the those listed below and to personnel in the PCC and ED. Without your invaluable support, our study would not have been possible!

Linda Alberti
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Clinical Instructor, Internal Medicine and Pediatrics section of Infectious Diseases

Dr. Paul McCarthy
Professor and Section Chief of Pediatrics

Dr. Harry Moscovitz
Assistant Professor of Surgery and Emergency Medicine
Adult ED

Cholera testing

Vibrio cholera testing was performed as part of the CDC Bioterrorism Preparedness project. The purpose of the project was to evaluate the specificity of two colorimetric assays for rapid diagnostic kits; the New Horizons Bengal Smart kit for *Vibrio cholerae 0139* and *Vibrio cholerae* 01. All samples tested were negative for *V. cholerae*.

Abstracts and Poster Presentations

1) S. Bell, L. Harris, C. Fitzgerald, J. Pruckler, C. Braden, S. C. Edberg. **Comparison of Stool Isolation of Campylobacter-like Organisms by a Hydrogen-Enriched Atmosphere Filtration Method and Conventional Isolation Technique.** Presented at the CT Infectious Disease Society Meeting in May 2004.

2) C. Braden, L. Harris, D. Torpey, J. Johnson, J. Whichard, K .Gay, S. Bell, S.C. Edberg. **Antibiotic Resistant Organisms Among Human Stool Normal Flora**. Presented at the CT Infectious Disease Society Meeting in May 2004.

3) J.M. Hirshon, L. Harris, R. Heimer, J. Heckendorf, J. Meek, V. Mai, S. Bell, S.C. Edberg, D. Torpey, J. Johnson, C Bopp, C. Braden. **Unexplained Diarrhea Sentinel Surveillance**. Presented at the CT Infectious Disease Society Meeting in May 2004.

4) R.D. Klein, S. Bell , S.C. Edberg **Genus Specific PCR and Partial 16s rRNA Gene Sequencing for the Direct Detection of *Campylobacter* Species in Human Feces**. Presented at the CAP Annual Meeting 2004.

5) R.D. Klein, S. Bell , S.C. Edberg. ***Campylobacter Concisus* in Unexplained Diarrhea: Identification by Genus Specific 16s Ribosomal Gene Sequencing**. Oral presentation at Academy of Clinical Laboratory Physicians and Scientists Annual Meeting 2004. Paul E. Strandjord Young Investigator Award winner.

6) R.D. Klein, S. Bell , L. Harris, S.C. Edberg. **Surveillance for *Listeria monocytogenes* in Unexplained Diarrhea**. Presented at the Association for Molecular Pathology Annual Meeting 2004.

7) R.D. Klein, C. Fitzgerald, J. Pruckler, S. Bell, S.C. Edberg, C. Braden. ***Campylobacter Concisus* in Unexplained Diarrhea**. Presented at American Society for Microbiology General Meeting 2004.