



# *Shigella flexneri* Associated with MSM & HIV

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**Vincy Samuel, MPH, PhDc  
Epidemic Intelligence Service Fellow  
Florida Department of Health**

**Bureau of Epidemiology  
Grand Rounds  
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# Introduction

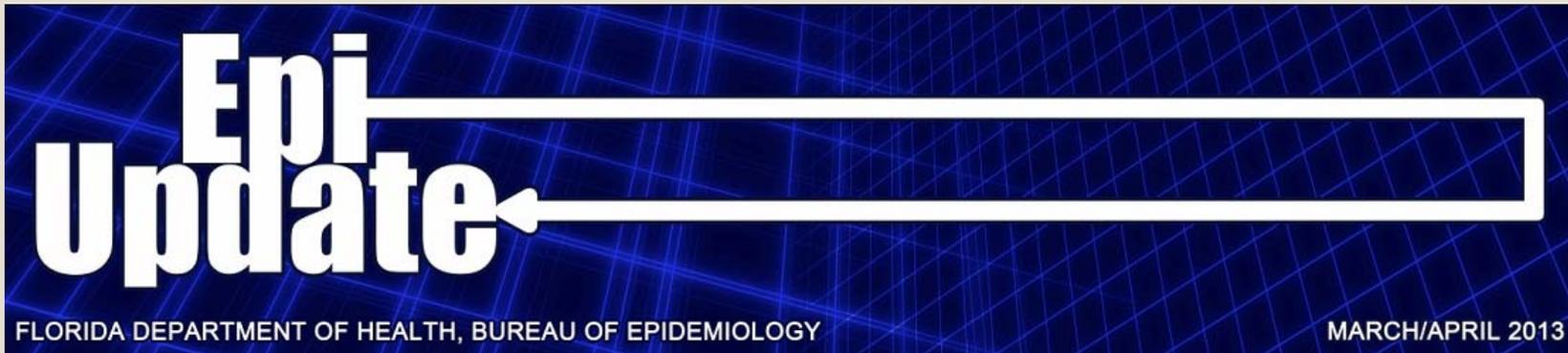
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- March 2012 → Florida Department of Health in Duval County, Epidemiology Program was notified of 3 persons with *Shigella flexneri* infections since January 2012 who had similar risk factors
- **Risk Factors**
  - Male
  - African-American
  - 25 – 30 years old
  - HIV positive
  - Contact with an ill sexual partner, close friend, or work contact



# Epi Update Article

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## *Shigella flexneri* Infections Among Men Who Have Sex with Men (MSM) and HIV – Duval County, 2012

*Rebecca Alcantara, R.N., B.S.N.*



# Background

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- Shigellosis is caused by a gram-negative bacillus in the family Enterobacteriaceae which primarily infects the distal small intestine and the large intestine
- 4 *Shigella* species with more than 40 serotypes
  - *sonnei* (86%)
  - *flexneri* (12%)
  - *dysenteriae* (<1%)
  - *boydii* (1%)
- Humans → natural host for *Shigella* bacteria



Table 1:  
Classification of *Shigella* subgroups

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Subgroup	Species	Number of Serotypes
A	dysenteriae	15
B	flexneri	8
C	boydii	19
D	sonnei	1



# Epidemiology

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- **Primary mode of transmission** → fecal-oral with person to person spread
  - Daycare settings
  - Among international travelers
  - Men who have sex with men (MSM)
  - People living in crowded conditions with inadequate sanitation
- **Symptoms**
  - Watery or bloody diarrhea
  - Abdominal pain
  - Malaise
  - Fever
  - Nausea
  - Vomiting
- **Incubation period** → 1 - 7 days (usually 1 - 3 days)
  - Self-limiting illness lasting between 4 - 7 days



# Case Definition of Shigellosis

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- **Clinical Description**

- Illness of variable severity characterized by diarrhea, fever, nausea, cramps, tenesmus

- **Lab Criteria**

- Confirmed = isolation of *Shigella* from a clinical specimen
- Suspect = detection of *Shigella* from a clinical specimen using a non-culture based method

- **Case Classification**

- Confirmed = case that meets confirmed laboratory criteria for diagnosis
- Probable = clinically compatible case that is epi linked to a confirmed case
- Suspect = case that meets suspect lab criteria for diagnosis



## Table 2: Lab Services for *Shigella* Isolates

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Lab Services	Description	Who/When
<b>Stool culture screening</b>	<ul style="list-style-type: none"> <li>Detect potential pathogens</li> </ul>	<ul style="list-style-type: none"> <li>Performed on all submitted stool specimen by BPHL</li> </ul>
<b>Pure isolate biochemical identification</b>	<ul style="list-style-type: none"> <li>Identification of pathogen</li> </ul>	<ul style="list-style-type: none"> <li>Performed on all <i>Shigella</i> isolates by BPHL</li> </ul>
<b>Serogroup identification</b>	<ul style="list-style-type: none"> <li><i>Shigella</i> isolates can be used for serogroup identification</li> </ul>	<ul style="list-style-type: none"> <li>Performed on all submitted <i>Shigella</i> isolates by BPHL</li> </ul>
<b>Serotyping</b>	<ul style="list-style-type: none"> <li>Subtyping method based on the immuno-reactivity of various antigens</li> </ul>	<ul style="list-style-type: none"> <li>Performed by CDC only when requested</li> </ul>
<b>Pulsed-Field Gel Electrophoresis (PFGE)</b>	<ul style="list-style-type: none"> <li>A laboratory method used to separate bacterial isolates into genetic fragments,</li> <li>Different fragments form a unique “DNA fingerprint” or band pattern for each bacterial isolate</li> </ul>	<ul style="list-style-type: none"> <li>Performed by BPHL when requested, particularly during outbreak investigations</li> </ul>



# PFGE

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- Technique frequently used to assist the surveillance and investigation of foodborne illness and outbreaks
- Creates a unique “DNA fingerprint” or PFGE pattern for disease-causing bacteria isolated from infected persons
- PFGE patterns can be compared at the local, state, and national levels to identify potential outbreaks and to focus the epidemiologic investigation of outbreaks
- PFGE patterns may be analyzed to determine if the patterns are similar or indistinguishable and may provide additional information during investigations
  - Finding isolates with the same PFGE pattern may be consistent with but does not prove a common source
  - Isolates with unrelated PFGE patterns presumptively came from different sources



# Incidence

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- Cyclical trend
- Duval County → fluctuates from less than 5 cases/year to 300-400 cases/year in 3-5 year cycles
- *S. sonnei*
  - Predominant species in the U.S. and Duval County
  - Historically → *S. sonnei* accounted for 72% of reported cases of shigellosis in the US with *S. flexneri* accounting for most of the remaining infections



# Literature Review



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Home > Canada Communicable Disease Report (CCDR) > CCDR 2005: Volume 31 > Outbreak of *Shigella flexneri* and *Shigella sonnei* enterocolitis in men who have sex with men, Quebec, 1999 to 2001

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## Outbreak of *Shigella flexneri* and *Shigella sonnei* enterocolitis in men who have sex with men, Quebec, 1999 to 2001

Source: Public Health Agency of Canada, 2005



# Goals of Study

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- To investigate possibility of *S. flexneri* and *S. sonnei* outbreaks in Quebec population among MSM
- To examine sensitivity of strains to 3 antibiotics
- To evaluate risk factors associated with *Shigella* spp. infections



## *Shigella* spp. Infections in Quebec, 2000-2001

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- 2000 → 2 cases of *S. sonnei* infection in MSM had been reported to DSP Montréal-Centre.
- 2001 → 147 cases of *Shigella* spp. infection were reported to DSP Montréal-Centre
  - 93 (63.3%) acquired the infection in Quebec
    - ✦ *S. sonnei* → 76.3%
    - ✦ *S. flexneri* → 21.5%
    - ✦ Other/unknown spp. → 2.2%
  - 66 of the 93 patients (71%) were men
  - 30 out of 37 men (81%) whose sexual orientation was known were MSM & 29 of them (96.7%) had an *S. sonnei* infection



## *S. flexneri* & *sonnei* in Quebec, 1999-2001

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- 1999 – 2001 → 76 cases of *Shigella* spp. infection were identified by medical micro & infectiology lab of a hospital
- December 1999 - December 2000 → *S. flexneri* serotype 3 was documented in seven men between ages of 26 & 40
  - Sexual orientation & HIV status were known for 2 patients → both were MSM & HIV positive
- February 2000 - December 2001 → *S. sonnei* strain was documented in 27 men between ages of 22 & 54
  - Sexual orientation was known for 5 patients → all were MSM
  - HIV status was known for 3 patients → 1 was HIV positive



## Antibiotic Sensitivity

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- Antibiotic sensitivity was known for 31 of the 34 epidemic *Shigella* spp. strains
- 100% of the strains were resistant to ampicillin & sensitive to ciprofloxacin
- 90.3% were resistant to trimethoprim/sulfamethoxazole



## SHORT REPORT

### Shifting dominance of *Shigella* species in men who have sex with men

R. RATNAYAKE<sup>1,2\*</sup>, R. ALLARD<sup>2,3</sup> AND P. A. PILON<sup>2,4</sup>

<sup>1</sup> Canadian Field Epidemiology Program (CFEP), Public Health Agency of Canada

<sup>2</sup> Public Health Department, Montréal Health and Social Services Agency, QC, Canada

<sup>3</sup> Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montréal, QC, Canada

<sup>4</sup> Department of Social and Preventative Medicine, University of Montréal, QC, Canada

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first published online 10 May 2012

#### SUMMARY

Historically, *Shigella sonnei* has dominated other *Shigella* species infection in men who have sex with men (MSM) in Montréal. In early 2010, Toronto reported increased proportions of the more pathogenic *S. flexneri*, HIV co-infection and MSM in shigellosis cases since 2009. Analytical methods were used to assess whether *S. flexneri* had dominated Montréal's MSM cases since 2009 and whether changes had occurred in notifications in MSM and HIV-co-infected MSM. *S. flexneri* increased by 6·7% per month since 2007 and predominated in MSM since 2009 without changes in HIV co-infection or similar shifts in the general population. The results suggest that the surveillance of *Shigella* species in order to detect species shifts is beneficial for surveillance, given the potential for increased transmission and severity of *S. flexneri* in HIV-positive MSM.

**Key words:** Men who have sex with men, outbreak, *Shigella*, surveillance.



## *S. sonnei* in Montreal

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- Montreal → *S. sonnei* has been responsible for person-to-person outbreaks within:
  - Day-care facilities
  - Religious communities
  - MSM



## *Shigella* and MSM

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- Elevated prevalence of HIV/AIDS immunosuppression in MSM compared to other population groups
- MSM experience increased carriage and transmission of *Shigella*
- Conditions favorable for outbreaks to occur



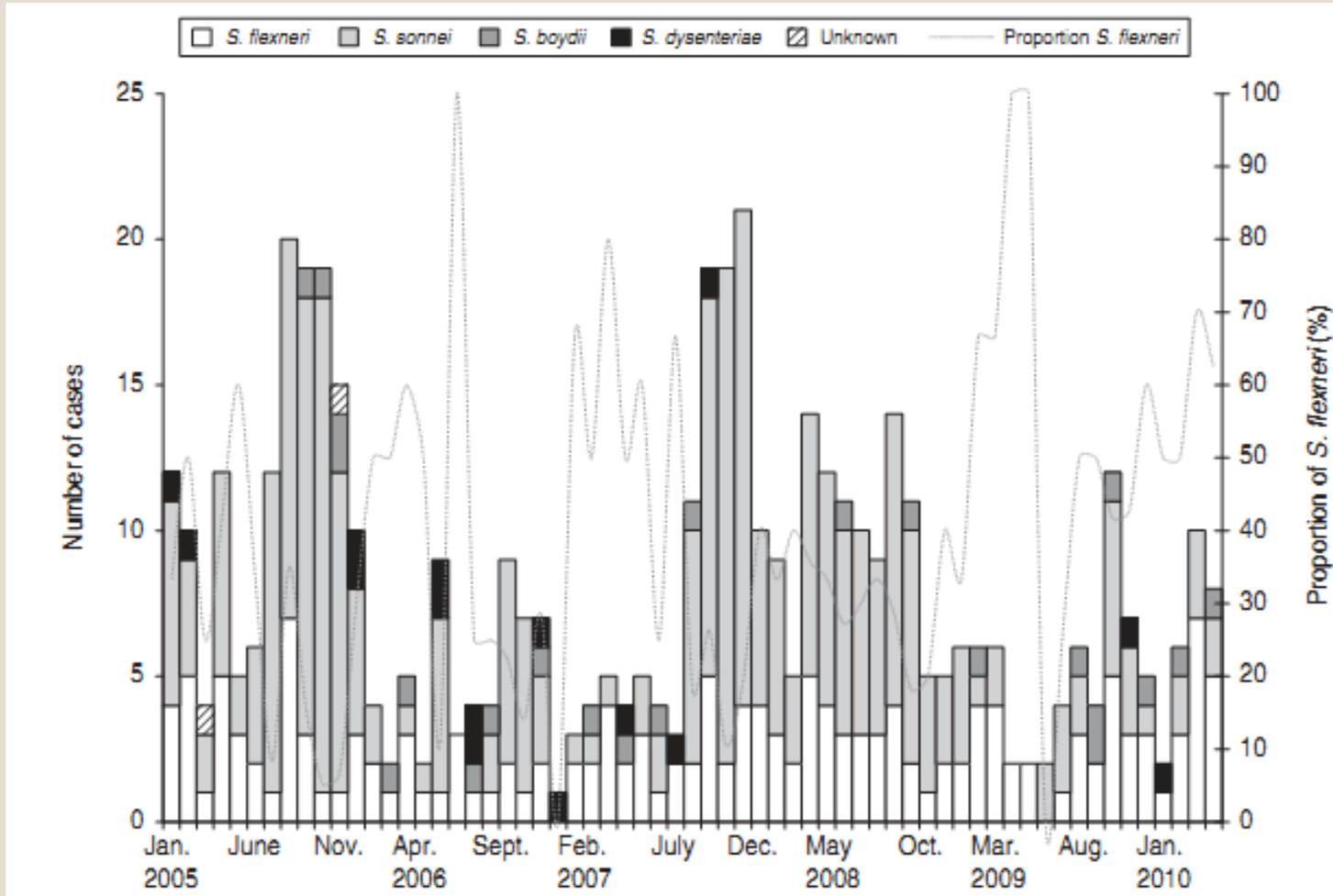
# *Shigella* and MSM

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- February 2010 → Public Health Department of Toronto reported:
  - Reversal from *S. sonnei* to *S. flexneri* (56% of cases notified in 2009)
  - Increase in MSM and HIV co-infection in shigellosis cases
- Epidemiological study
  - Discover whether shift to *S. flexneri* had recently taken place in MSM in Montreal
  - Determine impact on severity of disease



Figure 1: *Shigella* Cases by Species and Month, Montreal, 1/1/05-3/31/10



Source: Ratnayake, et. al., 2012



# MMWR<sup>TM</sup>

## Morbidity and Mortality Weekly Report

Weekly

August 26, 2005 / Vol. 54 / No. 33

### ***Shigella flexneri* Serotype 3 Infections Among Men Who Have Sex with Men — Chicago, Illinois, 2003–2004**

During 2003–2004, the Chicago Department of Public Health (CDPH) investigated an increase in reported *Shigella flexneri* serotype 3 infections among adult males. This report summarizes the investigation into those cases and underscores the potential for sexual transmission of enteric infections among men who have sex with men (MSM).



Figure 2: Number\* of *Shigella flexneri* serotype 3 cases, by demographic group – Chicago, Illinois, 1995-2004

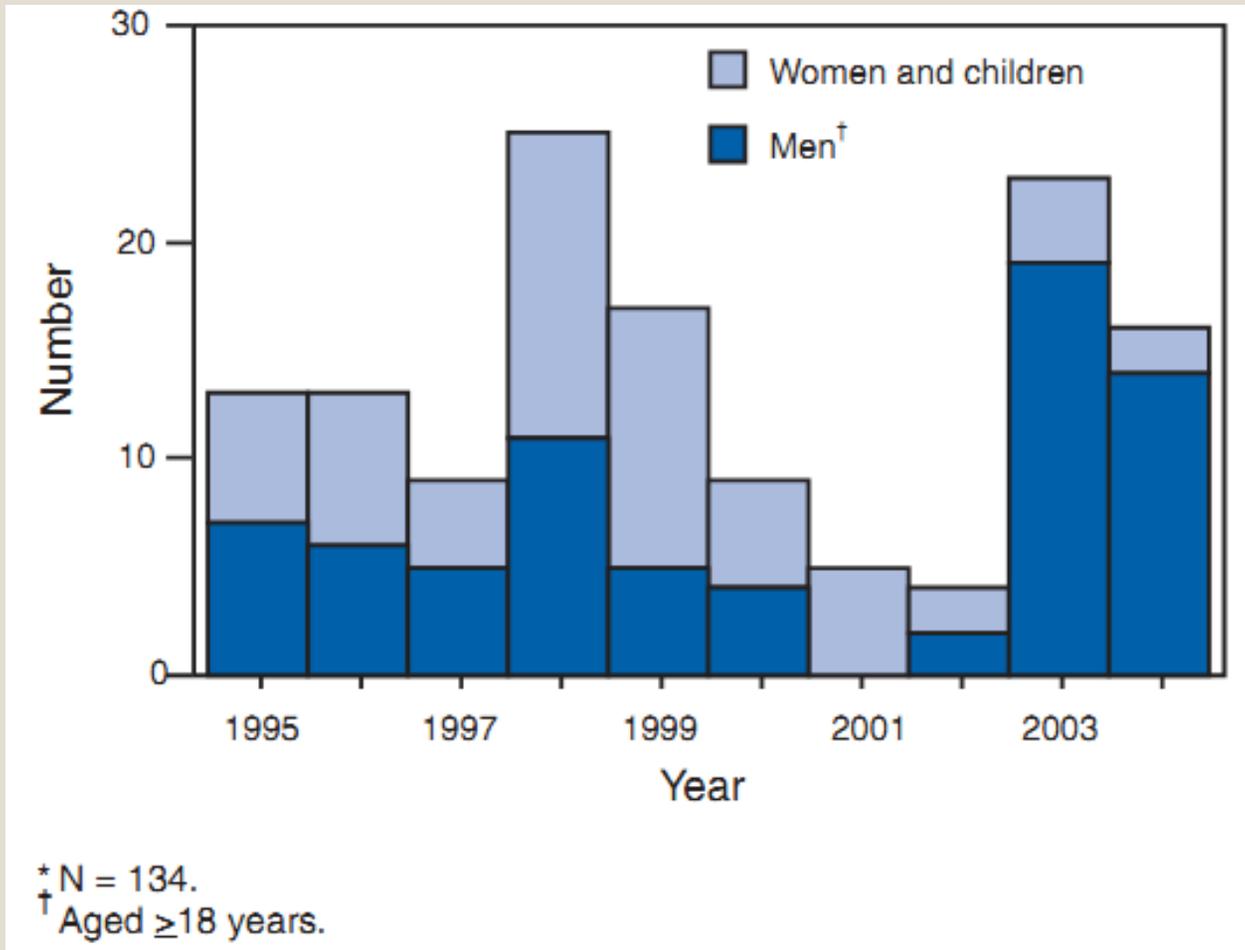
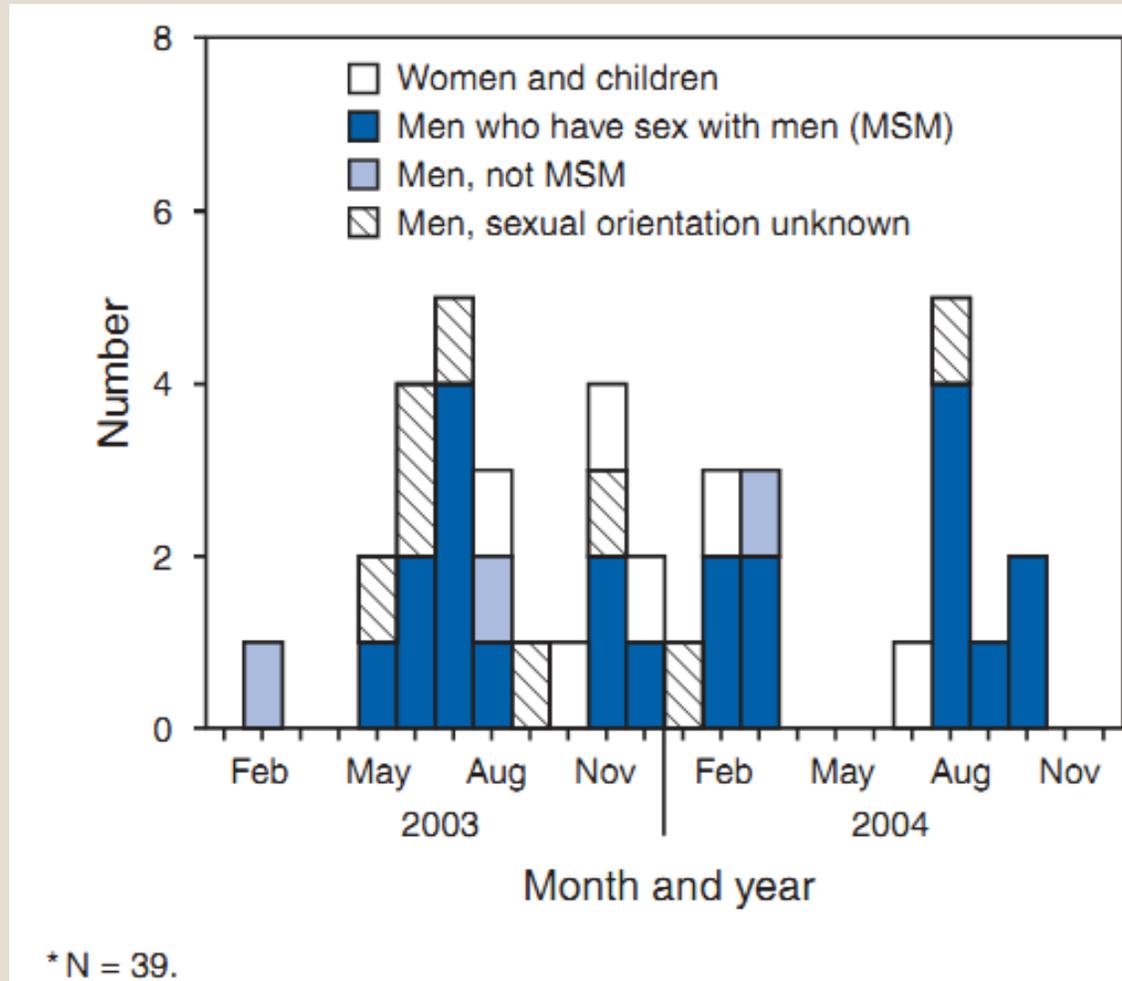




Figure 3: Number of *Shigella flexneri* serotype 3 cases, by sex, sexual orientation, and month of onset – Chicago, Illinois, 2003-2004





# Case-Control Study

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## Case-Control Study of Shigellosis in San Francisco: The Role of Sexual Transmission and HIV Infection

**Tomás J. Aragón,<sup>1,2</sup> Duc J. Vugia,<sup>3,4</sup> Sue Shallow,<sup>4</sup> Michael C. Samuel,<sup>4</sup> Arthur Reingold,<sup>2,4</sup> Frederick J. Angulo,<sup>5</sup>  
and Williamson Z. Bradford<sup>4</sup>**

<sup>1</sup>San Francisco Department of Public Health, City and County of San Francisco, San Francisco, <sup>2</sup>School of Public Health, University of California at Berkeley, Berkeley, <sup>3</sup>California Department of Health Services, Richmond, and <sup>4</sup>California Emerging Infections Program, Oakland, California; and <sup>5</sup>Centers for Disease Control and Prevention, Atlanta, Georgia



Table 3: Distribution of *Shigella* species & Clinical Symptoms

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Characteristic	Proportion (%) of patients
<i>Shigella</i> species infection	
<i>Shigella dysenteriae</i>	1/76 (1.3)
<i>Shigella flexneri</i>	40/76 (52.6)
<i>Shigella sonnei</i>	35/76 (46.1)
Symptoms	
Abdominal pain	69/76 (90.8)
Rectal urgency	64/76 (84.2)
Chills	62/75 (82.7)
Lightheadedness	50/76 (65.8)
Night sweats	46/73 (63.0)
Mucus in stool	43/70 (61.4)
Nausea	43/76 (56.6)
Tenesmus	42/76 (55.3)
Blood in stool	40/74 (54.1)
Vomiting	28/76 (36.8)

Source: Aragon, 2007



Table 4: Factors Associated with *Shigella* Infection

Variable	<i>S. flexneri</i>	<i>S. sonnei</i>	P
Male sex	36/40 (90.0)	22/35 (62.9)	.0063
MSM	36/36 (100)	12/22 (54.5)	<.0001
History of STD	27/40 (67.5)	14/35 (40.0)	.0213
HIV positive	22/40 (55.0)	6/35 (17.1)	.0008
Any sexual activity	26/40 (65.0)	18/34 (52.9)	.3463
Sexual anal contact			
No sexual activity and no anal contact	13/40 (32.5)	24/35 (68.6)	...
Sexual activity but no anal contact	5/40 (12.5)	4/35 (11.4)	...
Indirect anal contact <sup>a</sup>	10/40 (25.0)	4/35 (11.4)	...
Direct anal contact <sup>b</sup>	12/40 (30.0)	3/35 (8.6)	.0105
Foreign travel	1/40 (2.5)	16/35 (45.7)	<.0001
Diarrheal contact <sup>c</sup>	4/38 (10.5)	4/33 (12.1)	1.0000
Drinking unfiltered water <sup>d</sup>	1/40 (2.5)	4/29 (13.8)	.1537



**Table 5: Multivariable Unconditional Logistic Regression Models of Shigellosis by Risk Factors**

Model, variable	Case patients	Control subjects	OR (95% CI)	P
<b>A: men only</b>				
Foreign travel	11/58 (19.0)	7/110 (6.4)	20.0 (5.26–76.3)	<.0001
MSM	48/58 (82.8)	30/110 (27.3)	8.24 (2.70–25.2)	.0002
HIV positive	28/58 (48.3)	7/110 (6.4)	8.17 (2.71–24.6)	.0002
Sexual anal contact				
No anal intercourse	29/58 (50.0)	93/110 (84.5)	1.00 (reference)	...
Indirect anal contact <sup>a</sup>	14/58 (24.1)	13/110 (11.8)	1.57 (0.49–5.04)	.4489
Direct anal contact <sup>b</sup>	15/58 (25.9)	4/110 (3.6)	7.50 (1.74–32.3)	.0068
<b>B: women only<sup>c</sup></b>				
Foreign travel	7/18 (39.9)	0/36	21.0 (2.52–899)	.0002
<b>C: MSM only</b>				
Foreign travel	4/48 (8.3)	2/30 (6.7)	4.84 (0.74–31.8)	.1007
HIV positive	28/48 (58.3)	5/30 (16.7)	8.59 (2.56–28.9)	.0005
Sexual anal contact				
No anal intercourse	19/48 (39.6)	20/30 (66.7)	1.00 (reference)	...
Indirect anal contact	14/48 (29.2)	8/30 (26.7)	1.69 (0.50–5.76)	.3995
Direct anal contact	15/48 (31.3)	2/30 (6.7)	9.56 (1.69–54.0)	.0106

Source: Aragon, 2007



## *S. flexneri* in Duval County, 2012

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- Literature search demonstrated an association between *S. flexneri* infection and HIV/MSM
- FDOH in Duval County, Epidemiology Program began monitoring this association locally



# Method

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- Data was gathered from Merlin to document the number of reported shigellosis infections during the last 10 years
- Merlin
  - Reportable disease surveillance software for FDOH Bureau of Epidemiology
  - Storage, transmission, & analysis of reportable disease case reports



# Method

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- Risk factor & exposure data was gathered through client interviews & medical chart review
- PFGE was performed by the Bureau of Public Health Laboratories (BPHL) in Jacksonville on specimens forwarded to the BPHL for speciation
- A specimen in this group was forwarded to CDC for serotyping
- Length of hospitalization was gathered through medical chart review



**Table 6: Incidence & Percentage of Confirmed Cases of *Shigella* by Species in Duval County, 2002-2013**

<b>Year</b>	<b>Sonnei</b>	<b>Flexneri</b>	<b>Total</b>
2002	199 (98.0%)	4 (2.0%)	203
2003	237 (100%)	0 (0%)	237
2004	15 (83.3%)	3 (16.7%)	18
2005	2 (100%)	0 (0%)	2
2006	288 (99.7%)	1 (0.3%)	289
2007	81 (100%)	0 (0%)	81
2008	15 (88.2%)	2 (11.8%)	17
2009	2 (100%)	0 (0%)	2
2010	100 (95.2%)	5 (4.8%)	105
2011	59 (96.7%)	2 (3.3%)	61
2012	3 (18.8%)	13 (81.3%)	16
2013	32 (88.9%)	4 (11.1%)	36
<b>Total</b>	<b>1033 (96.8%)</b>	<b>34 (3.2%)</b>	<b>1067</b>



Figure 4: Percentage of Confirmed Cases of *Shigella* by Species in Duval County, 2002-2013

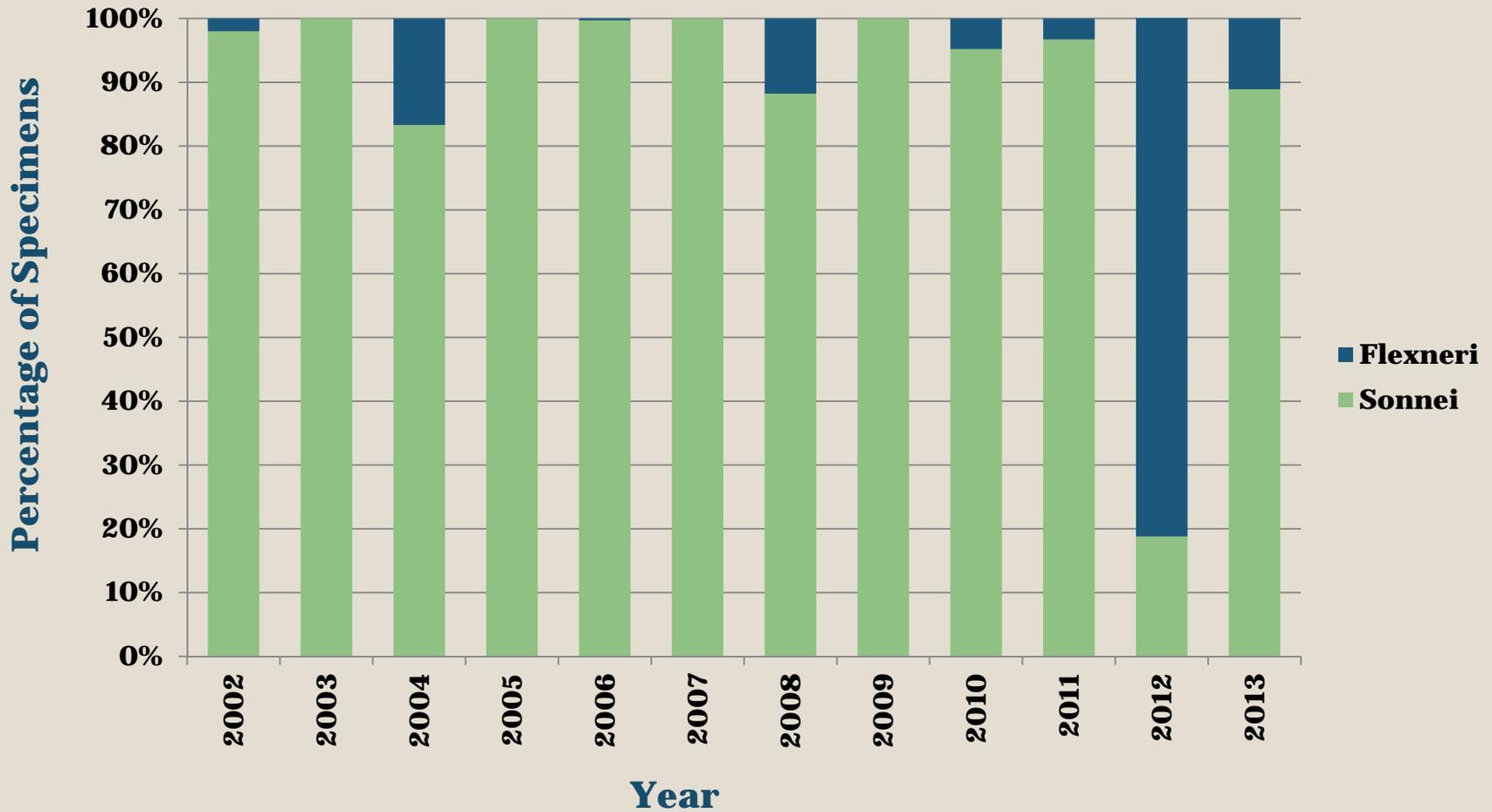
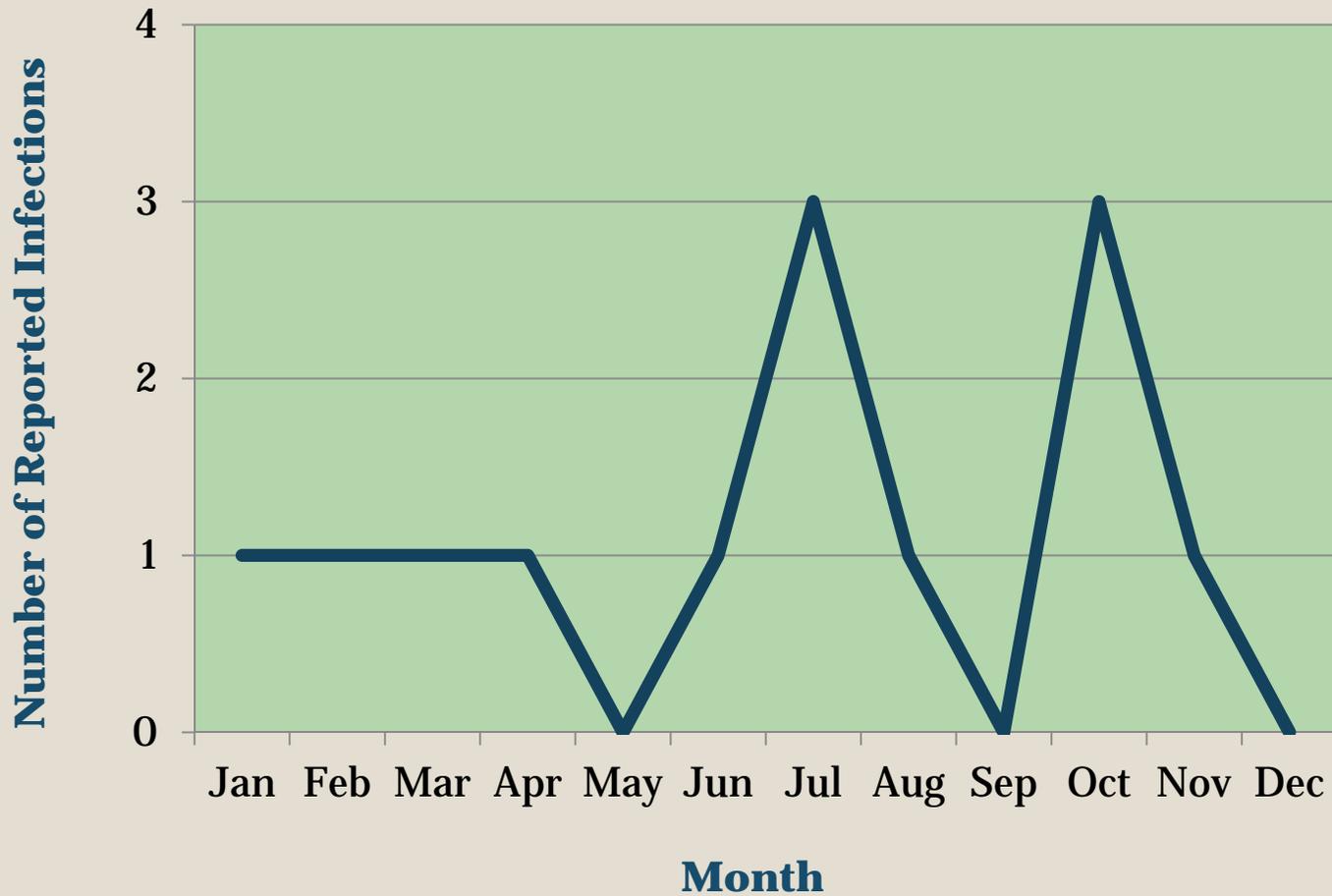




Figure 5: Incidence of Reported *Shigella flexneri* Infections by Month, Duval County, FL, 2012

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## *S. flexneri* Cases in 2012

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- 13/18 reported shigellosis infections were *S. flexneri*
- Risk Factors
  - Men → 11/13 (84.6%)
  - African American → 10/13 (77.0%)
  - Age between 18 & 35 years → 9/13 (69.0%)
  - HIV infection and/or MSM → 10/13 (77.0%)
- Primary reason for ED visit/admission → gastrointestinal symptoms (100%)
- Bloody diarrhea → 6/13 (46%)
- PFGE was done on 5 specimens and all 5 matched



## Hospitalization of 2012 *S. flexneri* Cases

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- 2 children visited the hospital ED but were not admitted
- The other 11 infected persons
  - Hospitalized between 3 - 25 days (median = 4 - 5 days)
  - Longest hospital stay was associated with bloody diarrhea, recurrent UTIs, pyelonephritis, & untreated HIV/AIDS.



## Table 7: Comparison of Antimicrobial Susceptibilities with Matching PFGEs

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#	ONSET	BLOODY DIARRHEA	# of HOSPITAL STAY DAYS	RESISTANCE	PFGE DONE
1	1/3/2012	yes	25 days	trimeth/sulfa	
2	2/9/2012	no	4 days	trimeth/sulfa & ampicillin	
3	3/19/2012	yes	5 days	trimeth/sulfa	
4	4/18/2012	yes	7 days	trimeth/sulfa & ampicillin	
5	6/16/2012	no	4 days	sensitive to both	PFGE
6	7/9/2012	no	4 days	ampicillin	
7	7/12/2012	no	3 days	no sensitivity done	PFGE
8	7/31/2012	no	4 days	no sensitivity done	
9	8/29/2012	yes	5 days	trimeth/sulfa & ampicillin	
10	10/3/2012	yes	5 days	trimeth/sulfa & ampicillin	
11	10/8/2012	no	ED X 2	ampicillin	PFGE
12	10/28/2012	no	4 days	sensitive to both	PFGE
13	11/9/2012	yes	ED X 1	no sensitivity done	PFGE



## Table 8: Confirmed *Shigella* Cases, Florida, 1/1/13 – 5/30/13

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County	# of Confirmed Shigella Cases per County
Duval	42
Dade	23
Broward	21
Bay	18
Hendry	10
Escambia	8
Lee, Palm Beach	7
Okaloosa, Orange	6
Alachua, Citrus	4
Clay, Collier, Lake, Marion, Martin, Pasco, Walton	2
Columbia, Hernando, Highlands, Hillsborough, Indian River, Jackson, Levy, Manatee, Nassau, Osceola, Polk, Santa Rosa, Sarasota, Seminole, St. Johns, St. Lucie, Sumter, Volusia	1
Total	188

Source: Merlin



## Confirmed *Shigella* Cases, Duval County, 1/1/13 – 5/30/13

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- 42 cases of *Shigella*
  - *S. sonnei* – 38 cases (90%)
  - *S. flexneri* – 4 cases (10%)



## 2013 *S. flexneri* Cases, 1/1/13 – 5/30/13

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- **Risk Factors**
  - Male → 4/4 (100%)
  - African- American → 4/4 (100%)
  - 25 – 30 years old → 4/4 (100%), mean = 26.5 years
  - HIV positive → 2/4 (50%), with 1 of the 2 also being MSM
- **PFGE**
  - 2/4, both are matches to 2012 *S. flexneri* specimens with PFGE results
  - Both resistant to trimeth/sulfa & ampicillin



# Control of Outbreaks

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- Surveillance of *Shigella* and other STIs in MSM
- Prompt public health intervention
  - Increased attention to shifts in species can provide an early warning for intervention measures



# Recommendations

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- **To reduce risk for sexually transmitted enteric infections:**
  - Persons with diarrhea should refrain from oral-anal, oral-genital, and anal-genital contact while symptomatic
- ***Shigella* can be carried asymptotomatically**
  - Wash hands and anal-genital regions thoroughly with soap & water before & after sexual activity
- **Use of condoms during oral-genital or anal-genital contact, dental dams during oral-anal contact, and gloves during digital-anal contact**



# Recommendations

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- Clinicians should request appropriate lab exams (i.e. stool culture for patients with diarrhea who are MSM)
- Counsel patients about the risk for infection with enteric pathogens during sexual activity that could expose them to feces
- *Shigella* isolates should be routinely serotyped and molecularly subtyped by PFGE to assist in detection of outbreaks



# Recommendations

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- **Investigations of outbreaks of shigellosis and other enteric diseases among MSM**
  - Better characterize specific high-risk behaviors for transmission
  - Identify effective prevention measures
  - Clarify role of HIV infection and antiretroviral therapy in the sexual transmission of *Shigella*
- **Collaborate with CHD HIV experts for assistance with interviewing patients**



# Limitations

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- Duplicate cases in Merlin
- Must use “Labs by Serogroup/Serotype” in order to pull *Shigella* cases by species in Merlin. One case may have multiple labs, which requires de-duplication in outside software (i.e. Excel).
- Cannot export line list from Merlin into Excel



# Acknowledgements

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  - Paul Fiorella, PhD – Molecular Epidemiology and Diagnostics
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  - Sa'ad Zaheer, MD, MSPH, FACE - Epidemiology Director
  - Angela Morgan, RN, BSN - Lead Nurse
  
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  - Katherine McCombs, MPH - EIS Administrator
  - Danielle Stanek, DVM – Medical Epidemiologist
  - Carina Blackmore, DVM, PhD, Dipl. ACVPM – State Public Health Veterinarian & Interim State Epidemiologist



# References

American Academy of Pediatrics (2012). Red Book. 645-647.

Alcantara, Rebecca (2013). *Shigella flexneri* infections among men who have sex with men (MSM) and HIV – Duval County, 2012. Epi Update: 8-12.

Aragon TJ, Vugia DJ, Shallow S, Samuel MC, Reingold A, Angulo FJ, Bradform WZ (2007). Case-control study of Shigellosis in San Francisco: the role of sexual transmission and HIV infection

Bureau of Epidemiology (2011). Shigellosis. Disease/Condition Specific Case Report Forms. Retrieved from [http://www.doh.state.fl.us/disease\\_ctrl/epi/topics/crforms.html](http://www.doh.state.fl.us/disease_ctrl/epi/topics/crforms.html).

Kansas Department of Health and Environment Division of Health (2008). Foodborne illness and outbreak investigation manual. Page 19.



# References

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MMWR Weekly (2005). *Shigella flexneri* serotype 3 infections among men who have sex with men – Chicago, Illinois, 2003-2004. Centers for Disease Control and Prevention, 54(33); 820-822.

Public Health Agency of Canada (2005). Outbreak of *Shigella flexneri* and *Shigella sonnei* enterocolitis in men who have sex with men, Quebec, 1999 to 2001. Canada Communicable Disease Report, 31 (8).

Ratnayake R, Allard R, Pilon PA (2012). Shifting dominance of *Shigella* species in men who have sex with men. Epidemiol. Infect. 140; 2082-2086.

Washington State Health Department (2013). Shigellosis.

WHO Global Foodborne Infections Network (2010). Laboratory protocol: Serotyping of *Shigella* spp. Page 17.