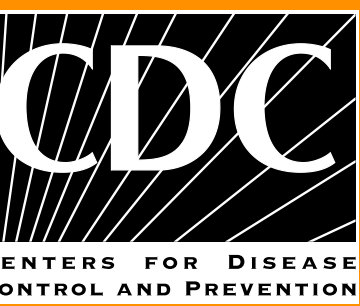


Genetic predictors of hemolytic uremic syndrome among persons infected with *Escherichia coli* O157

L. Hannah Gould¹, Timothy Jones², Jeffrey Canter³, Frederick Angulo¹, Elaine Scallan¹, and the CDC FoodNet Genomics Working Group

¹Centers for Disease Control and Prevention, Atlanta, GA; ²Tennessee Department of Health, Nashville, TN; ³Vanderbilt University, Nashville, TN



Abstract

Background and Objectives: Shiga toxin-producing *Escherichia coli* O157 (STEC O157) causes an estimated 73,000 illnesses annually in the United States, resulting in 2,168 hospitalizations and 61 deaths. Approximately 5-15% of persons infected with STEC O157 develop hemolytic uremic syndrome (HUS), the leading cause of renal failure in children. The Foodborne Diseases Active Surveillance Network (FoodNet) has launched a study to identify human genomic factors associated with the development of HUS among persons infected with STEC O157.

Methods: FoodNet conducts active, population-based surveillance for laboratory-confirmed STEC O157 infections at >650 laboratories serving the 10 FoodNet sites. All STEC O157 cases ascertained from 2006 to 2008 will be mailed a kit asking them to submit a mouthwash sample for DNA testing for polymorphisms and mutations in candidate genes. Medical chart review will be used to document if cases develop HUS, defined as a clinical diagnosis of HUS by a physician with the detection of STEC O157 as the causative agent by culture, serology, EIA, or PCR. The DNA profile of persons infected with STEC O157 who develop HUS will be compared to those without a HUS diagnosis to identify genetic variants.

Results: Study enrollment began in January, 2007 and will be conducted retrospectively for 2006. To date, 37 persons have been enrolled and an additional 40 persons have agreed to participate. Many FoodNet sites only recently received IRB approval, and as they have begun the number of specimens submitted is rising sharply. DNA has been successfully isolated from almost all specimens submitted thus far.

Discussion/Conclusion: This ongoing study is one of the first to look at human genomic factors associated with a food-borne pathogen. Identification of genetic factors associated with HUS will contribute to a better understanding of the pathogenesis of HUS and will have potential therapeutic and preventive implications.

Methods

Case Ascertainment

- ❖ Cases identified using several different mechanisms:
 1. Laboratory-confirmed STEC O157 cases ascertained in FoodNet
 2. HUS cases associated with STEC O157 infection reported to FoodNet's HUS surveillance system
 3. HUS cases associated with STEC O157 outbreaks in non-FoodNet sites

HUS Case Definition

- ❖ A clinical diagnosis of HUS by a physician, meeting the probable or confirmed national surveillance case definition for post-diarrheal HUS, and the detection of *E. coli* O157 as the causative agent by culture, serology, EIA, or PCR OR

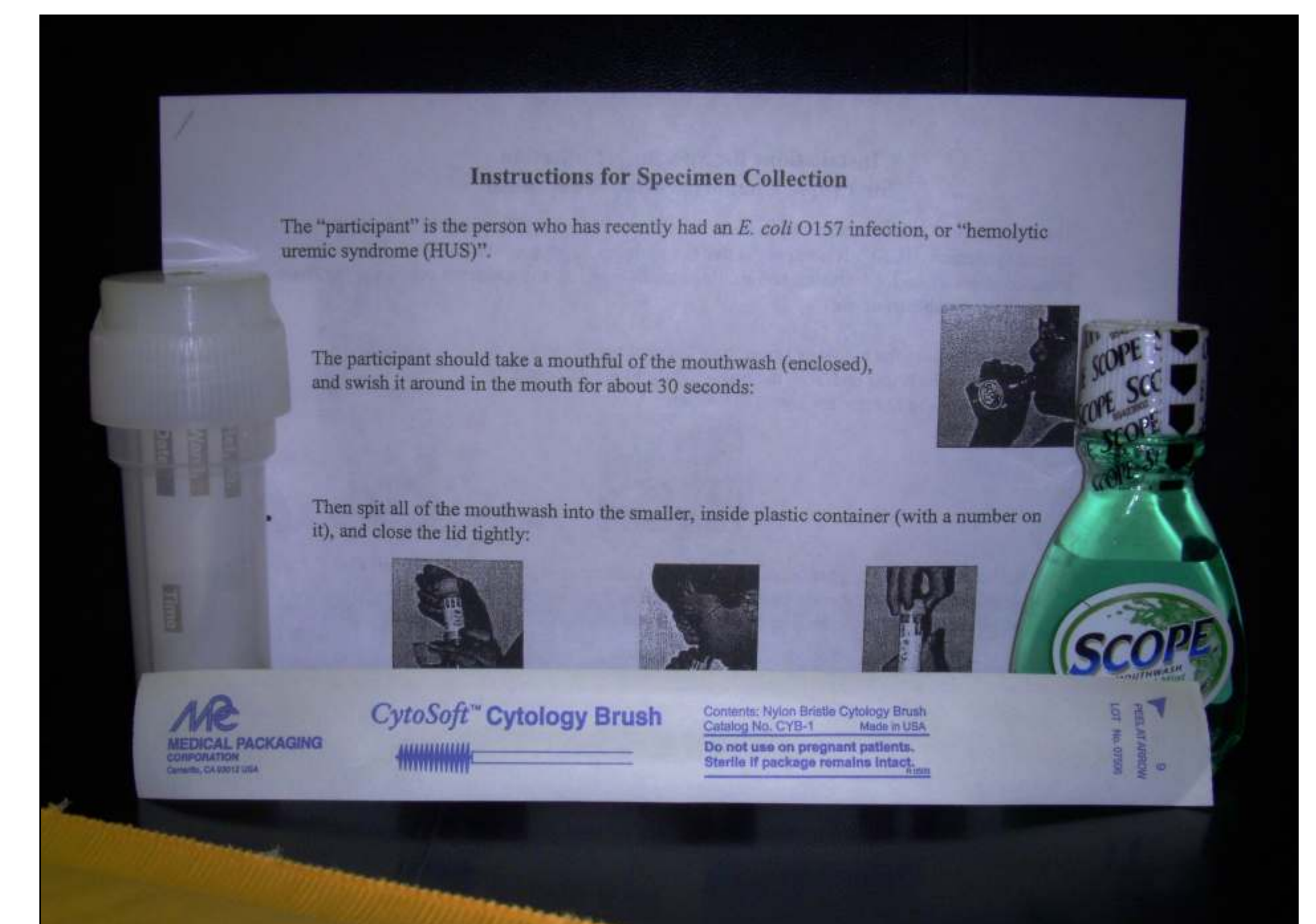
Note: CDC case definition for HUS requires evaluation of laboratory criteria including age- and sex-specific criteria for anemia with presence of erythrocyte fragments (i.e., schistocytes, burr cells, or helmet cells) on peripheral blood smear, platelet count <150,000/mm³, and renal insufficiency

Laboratory Testing

- ❖ DNA extracted from mouthwash samples and amplified
- ❖ Genetic analysis will be conducted when sufficient samples available
- ❖ Candidate genes to be analyzed include genes encoding:
 - ◆ complement factor H, I
 - ◆ IL-6, 8 and 10,
 - ◆ ENA-78,
 - ◆ G-CSF
 - ◆ Endothelin
 - ◆ CD46
 - ◆ von Willebrand factor cleaving proteinase ADAMS13

Case Interview and Testing

- ❖ Patients interviewed by state or local health department, consent obtained
- ❖ Kit sent to patient for collection and shipping of DNA specimens
 - ◆ Specimens obtained using mouthwash samples or buccal swab (Figure)
- ❖ Patients return samples in postage paid mailer to Vanderbilt University
- ❖ \$10 gift card to WalMart given to all participants



Background and Objectives

Escherichia coli O157

- ❖ *Escherichia coli* O157 (STEC O157) causes an estimated 73,000 illnesses, 2,168 hospitalizations, and 61 deaths in the U.S. each year
- ❖ 5-15% of persons infected with STEC O157 develop hemolytic-uremic syndrome (HUS)

Hemolytic Uremic Syndrome

- ❖ Leading cause of renal failure in children
- ❖ Triad of microangiopathic hemolytic anemia, thrombocytopenia, acute renal failure
- ❖ >80% of HUS cases ("typical") occur 2-3 weeks after STEC-associated gastroenteritis
- ❖ Non-infectious, familial ("atypical") HUS rarer
- ❖ Mortality rate of post-diarrheal HUS 3-5%

Role of Genomics— Evidence from Non-infectious HUS

- ❖ Increased
 - ◆ Interleukin (IL)-6, 8, 10
 - ◆ endothelin
 - ◆ -interferon
 - ◆ granulocyte-colony stimulating factor (G-CSF)
- ❖ Decreased
 - ◆ Neopterin
 - ◆ IL-2, 10
 - ◆ epithelial neutrophil activating peptide-78 (ENA-78)
 - ◆ C3
- ❖ Mutations in complement cascade proteins, von Willebrand factor cleaving protease gene

Objectives

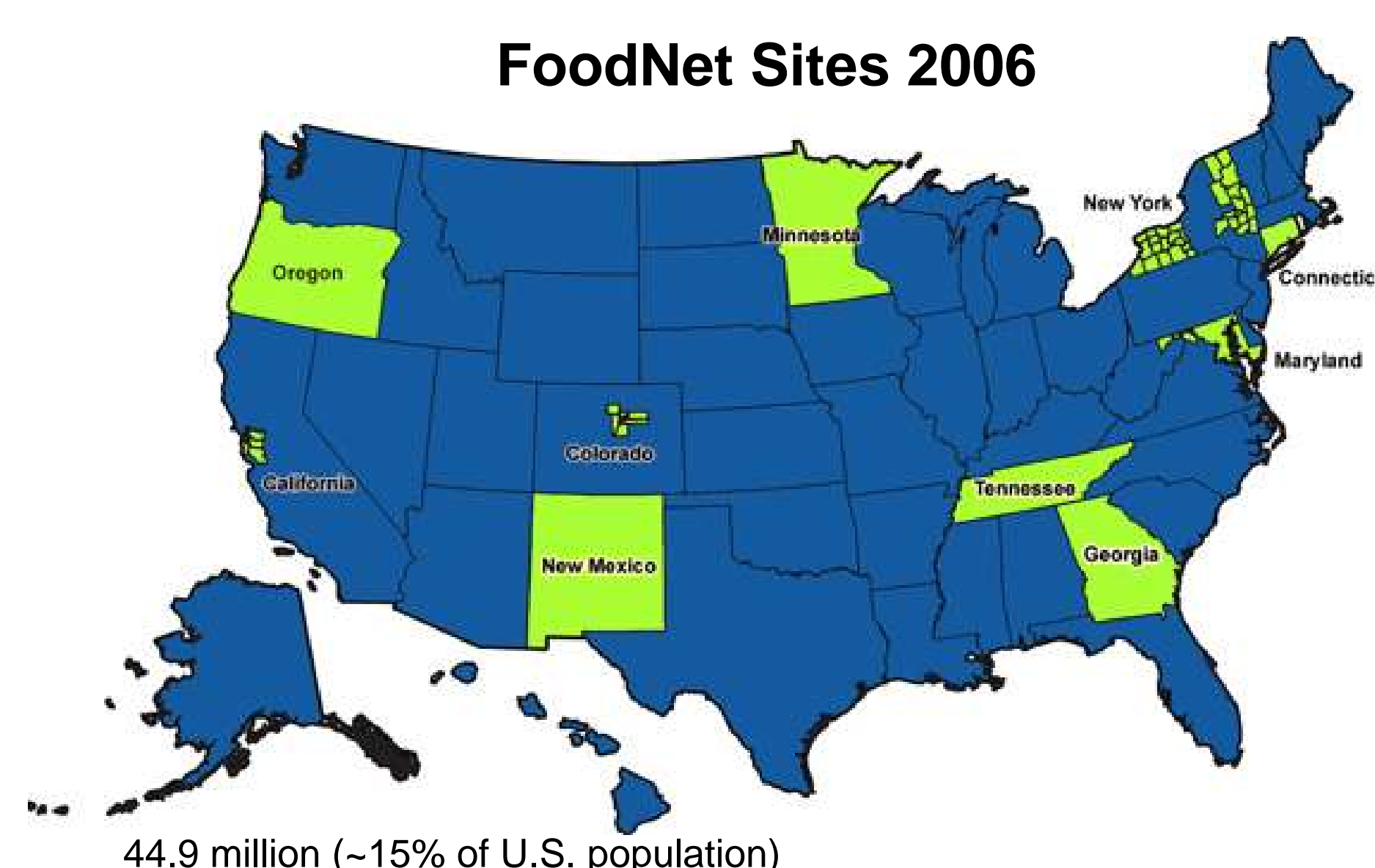
- ❖ To identify human genomic factors associated with the development of HUS among persons infected with STEC O157
- ❖ To calculate the proportion of disease risk attributable to genetic factors

FoodNet

What is FoodNet?

- ❖ Foodborne Diseases Active Surveillance Network
- ❖ Established in 1996
- ❖ Principal foodborne disease component of Emerging infections Program
- ❖ CDC, USDA-FSIS, FDA, and 10 participating state health departments

FoodNet Sites 2006



STEC O157 Surveillance

- ❖ Active, population-based surveillance for laboratory-confirmed STEC O157 infections
- ❖ FoodNet personnel routinely contact >650 clinical laboratories serving the FoodNet catchment area to ascertain cases

HUS Surveillance

- ❖ Network of pediatric nephrologists and infection control practitioners report pediatric (<18 years) HUS cases
- ❖ FoodNet personnel contact providers monthly to identify any unreported cases of pediatric HUS
- ❖ Hospital discharge data reviewed annually to ensure

Results

Enrollment by Site*

Site	Began Study	Enrolled	Not enrolled	Pending	Total Eligible
CA	4/1/2007	6	31	15	52
CT	7/15/2007	16	24	22	62
GA	3/1/2007	0	7	16	23
MD	4/19/2007	18	13	8	39
MN	11/1/2007	49	12	146	207
NM	4/27/2007	7	2	1	10
TN	12/13/2006	30	7	63	100
IN**	10/2006	6	--	--	6
Total		132	96	271	499

* CA, NY, and OR FoodNet sites have not yet received IRB approval
** Enrolled patients from STEC O157 outbreak

- ❖ Study began December, 2006
- ❖ Sites began enrolling cases as IRB approval obtained
 - ◆ 7 sites have begun enrolling cases
- ❖ Retrospective enrollment for 2006 STEC cases

Discussion/Conclusion

- ❖ Study ongoing through December 2009
- ❖ One of the first studies to look at human genomic factors associated with a food-borne pathogen
- ❖ Identification of genetic factors associated with HUS will contribute to a better understanding of the pathogenesis of HUS and will have potential therapeutic and preventive implications

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- Tennessee Department of Health
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- United States Food and Drug Administration-Center for Food Safety and Applied Nutrition
- Vanderbilt University