AMERICAN MEAT INSTITUTE

J. Patrick Boyle
President and CEO

August 18, 2010

The Honorable Tom Vilsack
Secretary
U.S. Department of Agriculture
Jamie L Whitten Federal Bldg.
Room 200-A
1400 Independence Avenue, SW
Washington, DC 20250

Dear Secretary Vilsack:

The American Meat Institute (AMI) looks forward to working with you and other government regulators in our efforts to continue the substantial progress that has been made in improving the safety of the nation's meat and poultry supply.

Food safety is AMI's top priority. Over the past 20 years, the meat and poultry industry has had significant success in reducing the pathogen risk profile of its products. Our members have instituted a non-competitive policy with respect to openly sharing food safety practices and knowledge, as well as supporting food safety research. The AMI Foundation has provided grants totaling more than $7 million to various institutions for the purpose of developing new food safety technologies that can be adopted by livestock producers, packers, processors and other food handlers.

One issue that is at the fore front of industry concern is the control of shiga-toxin producing *Escherichia coli* (STEC) including *E. coli* O157:H7. Substantial progress has been made in controlling *E. coli* O157:H7 in raw beef products, but AMI is concerned that the designation of non-O157:H7 STECs as adulterants will result in a misdirected regulatory program that will cause more harm than good. To that end, we are providing the following recommendations.

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Focus on Prevention

AMI supports President Obama's Food Safety Working Group recommendation that "food regulators shift towards prioritizing prevention and move aggressively to implement sensible measures designed to prevent problems before they occur." Declaring non-O157:H7 STECs to be adulterants in beef products does not fulfill President Obama's objective. Making a pathogen illegal through a policy change will not prevent this pathogen from occurring. Making non-O157:H7 STECs illegal also will needlessly divert scarce resources away from enhancing food safety prevention efforts when research shows that the intervention technologies we currently have in place are effective against various strains of *E. coli*.

Any new regulatory initiative must focus on developing and implementing effective process control programs to prevent rather than detect pathogens. The FSIS regulation to control *Listeria monocytogenes* in ready-to-eat meat and poultry products is an excellent example of the success that can be achieved by a regulatory approach that encourages implementation of effective preventive process control programs.

Regulatory programs that focus on testing as a way to eradicate pathogens from the food supply have historically been proven ineffective. Scientific experts worldwide know that testing cannot guarantee pathogens do not enter the food supply. That can only be done by using proven preventative measures that keep foodborne hazards from entering the food supply in the first place. Testing is useful to verify that food safety processing controls are working properly, but it is an ineffective tool for keeping hazards from entering the food supply. Safety cannot be tested into the product; safety must be built into the product. Any regulatory scheme that focuses on testing, instead of process control, will not make food safe.

AMI therefore recommends that any regulatory program that FSIS contemplates be addressed within the framework of the existing Hazard Analysis Critical Control Point regulation. USDA should commission a group of qualified experts to review the current science related to the development of a comprehensive farm-to-table preventative process control program for non-O157:H7 STECs in beef products and report their finding to USDA and other stakeholders. Such an examination could provide a practical means to establish quantifiable food safety objectives that can be used by USDA and the industry to improve public health.

Conduct a Comprehensive Public Health Risk Assessment

A better understanding of the public health issues associated with non-O157:H7 STECs is needed. Public health outbreaks associated with non-O157:H7 STECs in various foods have been documented, but no reported outbreak in the U.S. has been confirmed to be directly linked to beef products. [1] All stakeholders realize that such an outbreak could occur due to the endogenous presence of non-O157:H7 STECs in cattle, but many questions remain? In that regard, why have no confirmed outbreaks associated with beef products occurred in the U.S.? Why have non-O157:H7 STEC outbreaks occurred in other foods, but not in beef products? Why have non-O157:H7 STEC outbreaks associated with beef products occurred in other countries, but not in the U.S.? Does the pathogenicity of these microorganisms differ from *E. coli* O157:H7? Does the mere presence of the organism constitute a public health hazard?

AMI respectfully suggests that answers to such questions must be produced within the context of a comprehensive public health risk assessment that is subjected to public review before regulators embark on any regulatory program to control non-O157:H7 STECs in raw beef products.

Validate Analytical Laboratory Test Methods

At the present time, no relevant, validated, FSIS-accepted, rapid analytical test for non-O157:H7 STECs is commercially available. It is important to acknowledge that due to the limited time perishable beef products can be held and the logistics of holding products for several days pending cultural confirmation that non-O157:H7 STECs are present, a viable, rapid screening test is needed to make product dispositions. Therefore, an accurate, validated rapid analytical test must be available to the industry to effectively implement any regulatory program that would make it illegal to enter product containing non-O157:H7 STECs into commerce.

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[1] On August 11, 2010, the Enteric Disease Surveillance Coordinator for the North Dakota Department of Health, Medical Services Section and AMI reviewed the facts surrounding a foodborne disease outbreak that was suspected to be linked to ground beef. Information on the outbreak can be found at http://www.ndhealth.gov/disease/GI/Docs/Foodborne%20Outbreaks%20in%20ND%20updated%202009.pdf (Accessed August 10, 2010). The "suspect" ground beef product was cooked meatballs. The North Dakota Health Department was unable to confirm the suspected food source because no meatballs were available for testing. The meatballs were prepared the day before consumption at a private home wedding reception. No cooking temperatures were documented. The cooked meatballs were cooled at room temperature before transfer to the refrigerator for overnight storage. The temperature of the meatballs during cooling was not taken. The size of the container that was used to store the meatballs in the refrigerator was not determined. On the day of the reception, the meatballs and gravy were placed into a warming appliance, reheated and served. The North Dakota Health Department was able to test the gravy in which the meatballs were served and two macaroni salads. No other foods (*i.e.* side-dishes, salads, fresh produce) were tested. The gravy and the macaroni salads tested negative for shiga-toxin producing *E. coli*. Subsequently, the North Dakota Health Department declared ground beef to be the "suspect" food source because of temperature abuse and improper reheating.

Furthermore, accurate cultural confirmation tests must be available for regulatory purposes. It is our understanding that cultural confirmation tests are available for certain non-O157:H7 STEC serotypes, but not for all the serotypes that have been identified by USDA as a public health concern. Again, it is of paramount importance to have validated, peer-viewed cultural confirmation tests that are accepted by USDA before adopting a policy that beef products containing non-O157:H7 STECs be considered adulterated. Such confirmation tests must have an acceptable false positive and false negative rate for USDA to implement any regulatory program and for USDA to determine if commercially available screening tests are acceptable for use.

AMI strongly recommends that FSIS openly share with the meat and poultry industry, testing laboratories, and test kit manufacturers the sampling and analytical methods that the agency will use to implement any regulatory program and that the analytical methods are peer-reviewed before any regulatory program is initiated.

Conduct a Baseline Survey of Non-O157:H7 STECs on Beef Products

A better understanding of the prevalence of non-O157:H7 STECs related to beef products is needed. A limited amount of research has been conducted to assess the prevalence of non-O157:H7 STECs on beef products, but AMI has no knowledge of any research that has assessed quantitative levels of the pathogen on beef products. Furthermore, much of the prevalence survey work has been conducted by independent, private organizations and the data has not been published in peer-reviewed journals. AMI is not aware of any surveys that provide a validated, statistically balanced representation of the beef products produced in the U.S.

Furthermore, analytical methods to detect and quantify non-O157 STECs have not been standardized because no official USDA reference method is available. This creates a problem that also leads to widely varying interpretation of any prevalence data that has been previously collected and reported.

It is imperative that FSIS conduct a baseline survey of beef products to include beef carcasses, ground beef and the raw materials used to manufacture ground beef in order to assess the impact of any new regulatory program that the agency may be contemplating. The baseline survey design and sampling and analytical methods should be published for public comment to solicit the advice and counsel of scientific and technical experts before proceeding with any such survey.

Measure Progress Based on the Public Health Outcome

Many times food safety progress is erroneously measured by tasks performed, regulations published or other measurements that are not directly tied to a public health outcome. Regulatory or inspection activities that do not improve public health waste scarce resources and divert attention from issues of public health importance.

Food safety progress is most properly assessed by accurately measuring human health outcomes via illnesses, hospitalizations and deaths that are attributed to foodborne disease. For example, the Department of Health and Human Service's Healthy People 2010 goal of a 50 percent reduction in illnesses associated with key foodborne pathogens from 1997 illnesses levels provides an appropriate benchmark for evaluating progress for all food, but it needs to be refined in order to focus on the specific foods that are causing the illnesses.

If FSIS decides to further regulate non-O157:H7 STECs, we must point out that it is ordinarily prudent to evaluate the success or failure of any such initiative by actual illness reductions. In the case of beef, however, this is nearly impossible given that no non-O157:H7 STECs illness outbreaks have been confirmed in the U.S. This lack of documented illnesses is remarkable given that approximately 95 percent of the public health laboratories reported in a recent survey that they are screening for non-O157:H7 STECs. If regulatory efforts to reduce non-O157:H7 STECs in beef products cannot generate measurable, positive public health outcomes, the underlying point of the exercise must be drawn into serious question.

Expedite Approval of New Microbial Interventions

Over the past 15 years, the meat industry has spent millions of dollars researching and developing new technologies to eliminate or reduce STECs on beef products. In fact, the AMI Foundation has provided grants to the USDA's Agricultural Research Service to investigate whether certain microbial interventions currently known to be effective against *E. coli* O157:H7 are also effective antimicrobial treatments for non-O157:H7 STECs.

Many new microbial intervention technologies have been successfully implemented in beef processing facilities. Other highly effective interventions have not been implemented by the meat industry due to a lack of government approval of such technologies. New technologies are generally widely adopted by the industry if they are proven effective.

Specifically, approvals for carcass surface irradiation, bacteriophage use during various phases of production, feed additives such as chlorates, and other innovative technologies have not been approved for various reasons. Those reasons involve disputes over which regulatory agency has jurisdiction, the data that various regulatory agencies

require for approval and a general unwillingness by the federal government to actively assist in the approval process.

AMI recommends that USDA convene a joint task force of all federal agencies that are involved in the approval of new microbial intervention technologies and the affected meat and poultry industry to identify approval roadblocks and to develop a better, expedited approval process that can rapidly move new technology to commercialization. New preventive technologies that are effective against all STECs are needed to control these pathogens before USDA considers making non-O157:H7 an adulterant on beef products.

Determine Impact on International Trade

A policy change to make non-O157:H7 STECs an adulterant on beef products will significantly impact international trade. Such a policy shift will be viewed by our trading partners as erecting a non-tariff trade barrier to prevent entry of beef products into the U.S. The U.S. can expect reciprocal actions by importing countries that will have the effect of curtailing U.S. beef exports.

Any policy change contemplated by USDA must be considered in the context of the global beef market. Imposition of new regulatory mandates can have several unintended consequences that should be carefully considered before any policy changes are implemented.

AMI recommends that USDA, the U.S. Trade Representative, and the Department of State commission a study to determine the impact on international beef trade that would result from declaring non-O157:H7 STECs an adulterant on beef products.

Provide an Open and Transparent Public Policy Process

Any decision to implement new regulatory initiatives to control non-O157:H7 STECs in beef products must be informed through an open and transparent public policy process. Any new regulatory program to control non-O157:H7 STECs will likely impose significant financial and regulatory burdens on the meat industry, particularly if it involves declaring non-O157:H7 STECs to be adulterants. Such a decision will dictate more testing programs, additional operating costs, losing product value if it tests positive, and other inherent costs that must be weighed against any public health benefit.

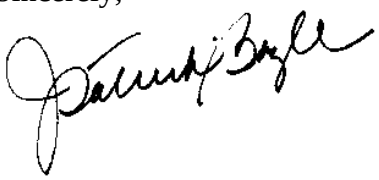
AMI recognizes that additional costs to control non-O157:H7 STECs, not only in beef products but other food products, including those regulated by FDA, may be appropriate if such costs are outweighed by corresponding public health benefits. At present, however, it is not obvious to many leading scientific experts that regulating non-O157:H7 STECs will result in substantial public health benefit, particularly given the available scientific evidence that microbial interventions that are used to control *E. coli* O157:H7 are effective in controlling non-O157:H7 STECs.

Associated legal questions must also be addressed. Prior to publication of the Hazard Analysis and Critical Control Point regulation, FSIS declared that *E. coli* O157:H7 was an adulterant based on an exception to the prevailing regulatory paradigm that raw meat and poultry products containing pathogens are not adulterated. FSIS took such regulatory action as a direct response to unique circumstances where the consumption of undercooked ground beef had resulted in illnesses and deaths caused by the presence of *E. coli* O157:H7. Given the absence of non-O157:H7 STEC illness outbreaks linked to beef, it is not readily apparent that there is an equally compelling reason to declare non-O157:H7 STECs as adulterants under present circumstances.

AMI recognizes that non-O157:H7 STECs in beef products may be a reason for potential public health concern, but the facts do not indicate that they pose a public health emergency. Therefore, AMI strongly recommends that if FSIS decides to further regulate non-O157:H7 STECs in beef products, it should only be done through notice and comment rulemaking. The questions surrounding non-O157:H7 STECs demand a disciplined, open, and transparent regulatory process.

Thank you for considering our views. We believe our recommendations have substantial merit and we would appreciate the opportunity to discuss them with you at your earliest opportunity.

Sincerely,



J. Patrick Boyle

cc: Jerry Mande, Deputy Under Secretary for Food Safety
Al Almanza, Administrator, Food Safety and Inspection Service

AMI Foundation
AMERICAN MEAT INSTITUTE

November 17, 2010

Elisabeth A. Hagen, M.D.
Under Secretary for Food Safety
U.S. Department of Agriculture
Jamie L. Whitten Building
12th & Jefferson Drive, SW
Room 227E
Washington, DC 20250-3700

Dear Under Secretary Hagen:

Shiga toxin-producing *Escherichia coli* (STEC) has and continues to be a significant public health concern to both the industry and the U. S. Department of Agriculture. As the American Meat Institute (AMI) Foundation begins the process of evaluating research proposals for funding in 2011 and the Department considers additional beef safety regulatory initiatives, I wanted to make you aware of selected research that is under way at the Foundation regarding non-O157:H7 STECs.

Starting more than a decade ago, the AMI Foundation's food safety program set a clear goal to reduce and ultimately eliminate *E. coli* O157:H7 in fresh beef, *Listeria monocytogenes* in ready-to-eat meat and poultry products, and *Salmonella* in meat and poultry products.

Since 1999, 25 research projects funded by the Foundation totaling $2 million have focused on *E. coli* in beef products. These projects have helped develop new technologies to reduce microbial hazards in beef products and to gain a better understanding of the taxonomy of microorganisms to select or create innovative antimicrobials for industry use.

The Foundation's research priorities are developed through a collaborative process of industry, academic and government experts who help solicit proposals for applied and fundamental research that will improve the microbial profile of meat and poultry products. The research advisory committee identifies knowledge gaps and predicts future research needs for the meat and poultry industry.

That is why in 2006, the Foundation began to include the non-O157:H7 STECs in their research priorities. This culminated in 2009 with the funding of AMIF's first research project dealing with sources of non-O157 STECs. As FSIS has narrowed its focus on specific STEC strains in addition to O157:H7, so too has the Foundation. This decision was the next logical step to better understanding how *E. coli* O157:H7 and STEC colonize in the same live animal, potentially contaminate the same products, and to determine if the same antimicrobial interventions work equally well for all strains.

During the evaluation of our 2009-2010 request for proposals, it became evident that we needed a more focused approach to food safety research on STECs. As a result, a special supplemental request for proposals was distributed to the research community in January 2010. This has led to funding at three institutions:

- *Antimicrobial interventions/application methods for the reduction of* E. coli *O157:H7 and* Salmonella *in beef trimming and/or ground beef* - University of Arkansas, Safe Foods International
  The main focus of this research is to utilize and validate antimicrobial properties of peroxyacetic acid, novel organic acids alone or in combination with a non-ionic surfactant on beef trimmings against *E. coli* O157:H7 O26, O103, O111, O121, O45, and O145 and *Salmonella* Typhimurium DT 104, Newport MDR-AmpC.

- *Evaluation of chemical decontamination treatments for beef trimmings against* E coli *O157:H7, non-O157 shiga toxin-producing* E. coli *and antibiotic resistant and susceptible* Salmonella *Typhimurium and* Salmonella *Newport* - Colorado State University
  The objective of the proposed study is to determine whether interventions known for reducing *E. coli* O157:H7 contamination on beef trimmings are also effective against *E. coli* O157:H7, non-O157 STEC (O26, O45, O103, O111, O121, and O145), and parent and derived *Salmonella* Typhimurium and *Salmonella* Newport strains.

- *Efficacy of commonly used antimicrobial compounds on decontamination of Shiga toxin-producing* E. coli *serotypes O45, O121, and* Salmonella *inoculated fresh meat* - USDA-ARS-U.S. Meat Animal Research Center
  The overall objective is to validate effectiveness of antimicrobial compound treatments on inactivation of STEC and *Salmonella* (MDR versus non-MDR strains) inoculated fresh beef. This study will complete other ARS work by adding the other two non-O157 STECS from the CDCs top six and include MDR and non-MDR *Salmonella* Typhimurium and Newport.

- *Evaluating the Efficacy of Commonly used Antimicrobial Interventions on Shiga-toxin Producing* E. coli *Serotypes O26, O103, O111, O145 and O157* - USDA-ARS-U.S. Meat Animal Research Center
  This research intends to validate the effectiveness of hot water, lactic acid, peroxyacetic acid, and other commercial antimicrobials on the inactivation of STEC inoculated fresh beef.

Final reports for these projects are due in the Summer 2011 and Summer 2012.

The Foundation believes it is essential to communicate the results of food and agricultural research transparently to improve food safety during meat and poultry production. That is why all final reports of AMI Foundation funded research are made available on our website (www.amif.org) for interested stakeholders.

In addition to research, the Foundation and AMI members believe food safety is further improved through adoption of recommended practices and education programs. Recognizing a need, AMI members developed a best practices document for consideration when manufacturing retail ground beef patties.

These recommendations led to a member-initiated request to develop educational programs for the beef industry. A curriculum, focusing on the safe production of ground beef, was developed by AMI member experts. The inaugural workshop will be held on February 2-3, 2011 in Kansas City. The Foundation anticipates this workshop being as successful as other member - driven workshops like the Advanced *Listeria monocytogenes* Intervention and Control workshop, which has been held frequently during the last 10 years to sold-out crowds.

I hope the enclosed information is helpful to you and the agency. The Foundation and AMI members recognize the potential public health implications of having shiga toxin-producing *E. coli* in the food chain. We believe our investment in research and the meat industry's proactive actions demonstrate our dedication in providing the safest product possible.

We will continue to update you on the Foundation activities and reports. Should you have any questions about the AMI Foundation and our research programs, please do not hesitate to contact me.

Sincerely,

James H. Hodges
President

cc: J. Patrick Boyle

Annual Foodborne Illness Estimates

	Known Foodborne Pathogens[1]	Unspecified Agents[2]	Total
Illnesses	9.4 million	38.4 million	47.8 million
Hospitalizations	55,961	71,878	127,839
Deaths	1,351	1,686	3,037

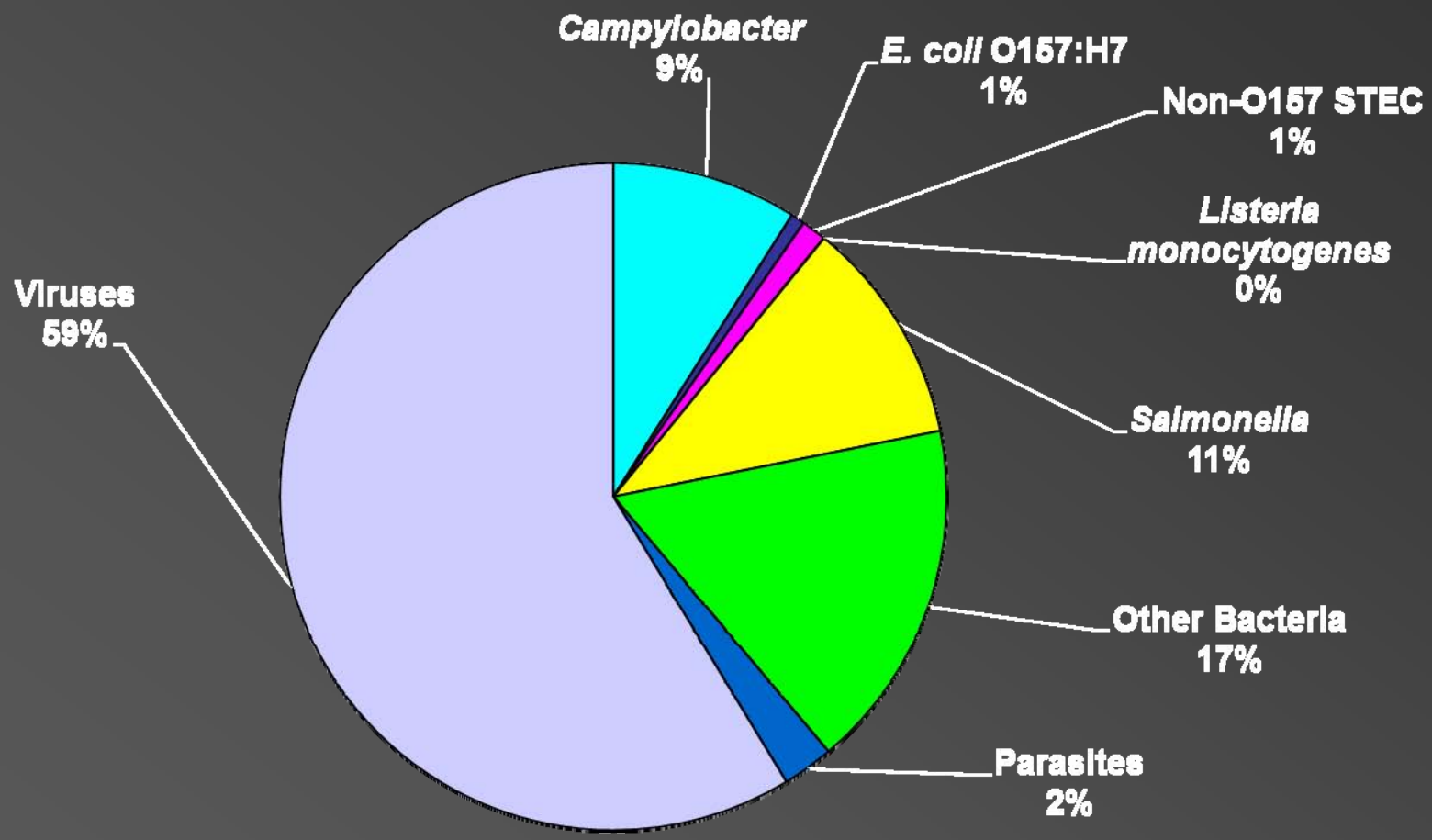
Total Illnesses from All FSIS Regulated Products[3]	584,335

[1] Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, Jones JL, and Griffin PM. (2011). Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis. 17(1): 7-15.

[2] Scallan E, Griffin PM, Angulo FJ, Tauxe RV, and Hoekstra RM. (2011). Foodborne illness acquired in the United States—unspecified agents. Emerg Infect Dis. 17(1): 16-22.

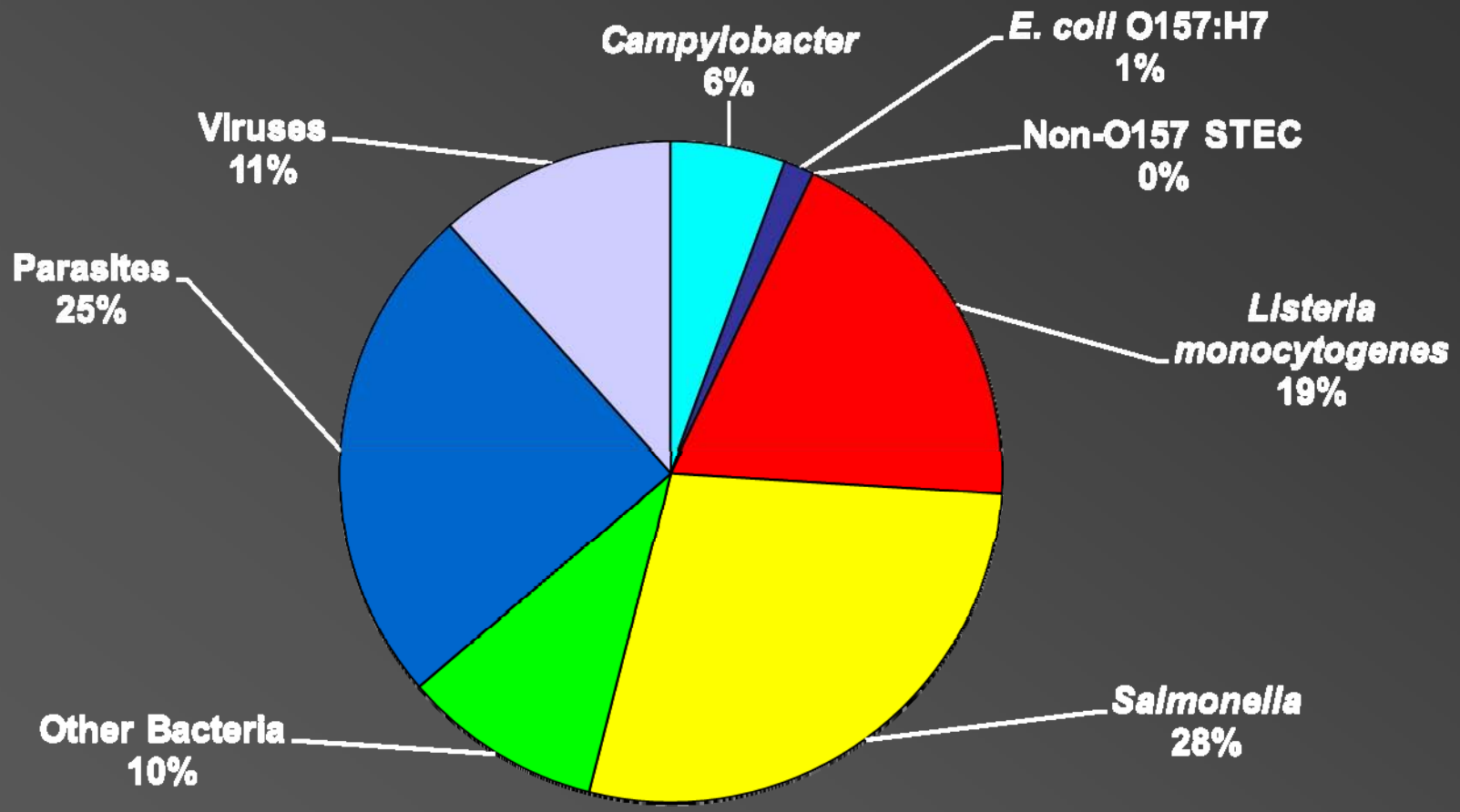
[3] USDA FY 2012 Rudget Summary and Annual Performance Plan.

Illnesses Attributed to Foodborne Transmission of Known Pathogens



Source: Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M A, Roy SL, Jones JL, and Griffin PM. (2011). Foodborne illness acquired in the United States major pathogens. Emerg Infect Dis. 17(1): 7 15.

Deaths Attributed to Foodborne Transmission of Known Pathogens



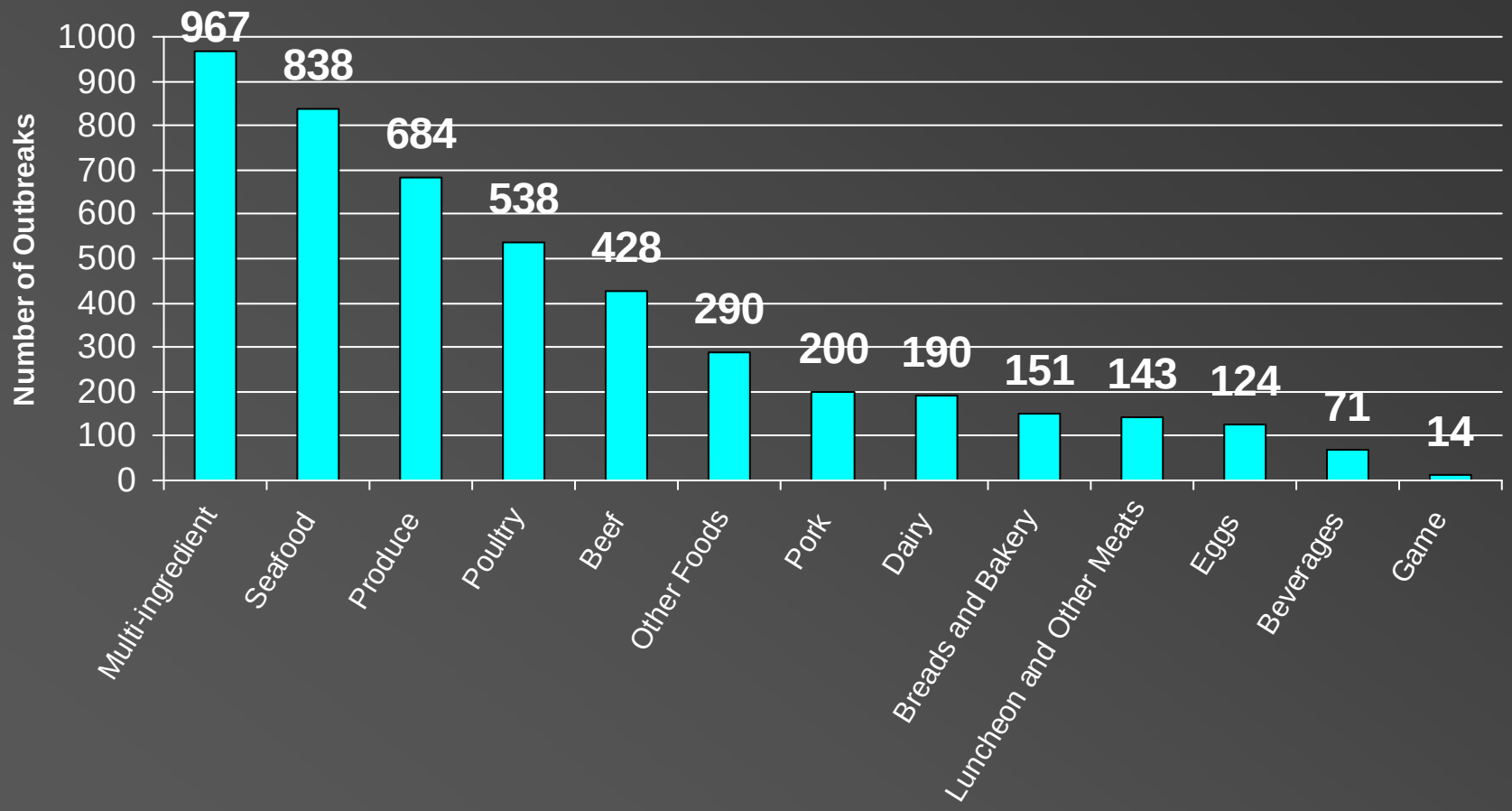
Source: Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M A, Roy SL, Jones JL, and Griffin PM. (2011). Foodborne illness acquired in the United States major pathogens. Emerg Infect Dis. 17(1): 7 15.

Comparison of O157 and Non-O157 STEC

	O157	Non-O157
Illnesses	63,153	112,752
Hospitalizations	2,138	271
Hospitalization Rate	46.2%	12.8%
Deaths	20	0
Death Rate	0.5	0.3
Travel Related	8%	4%

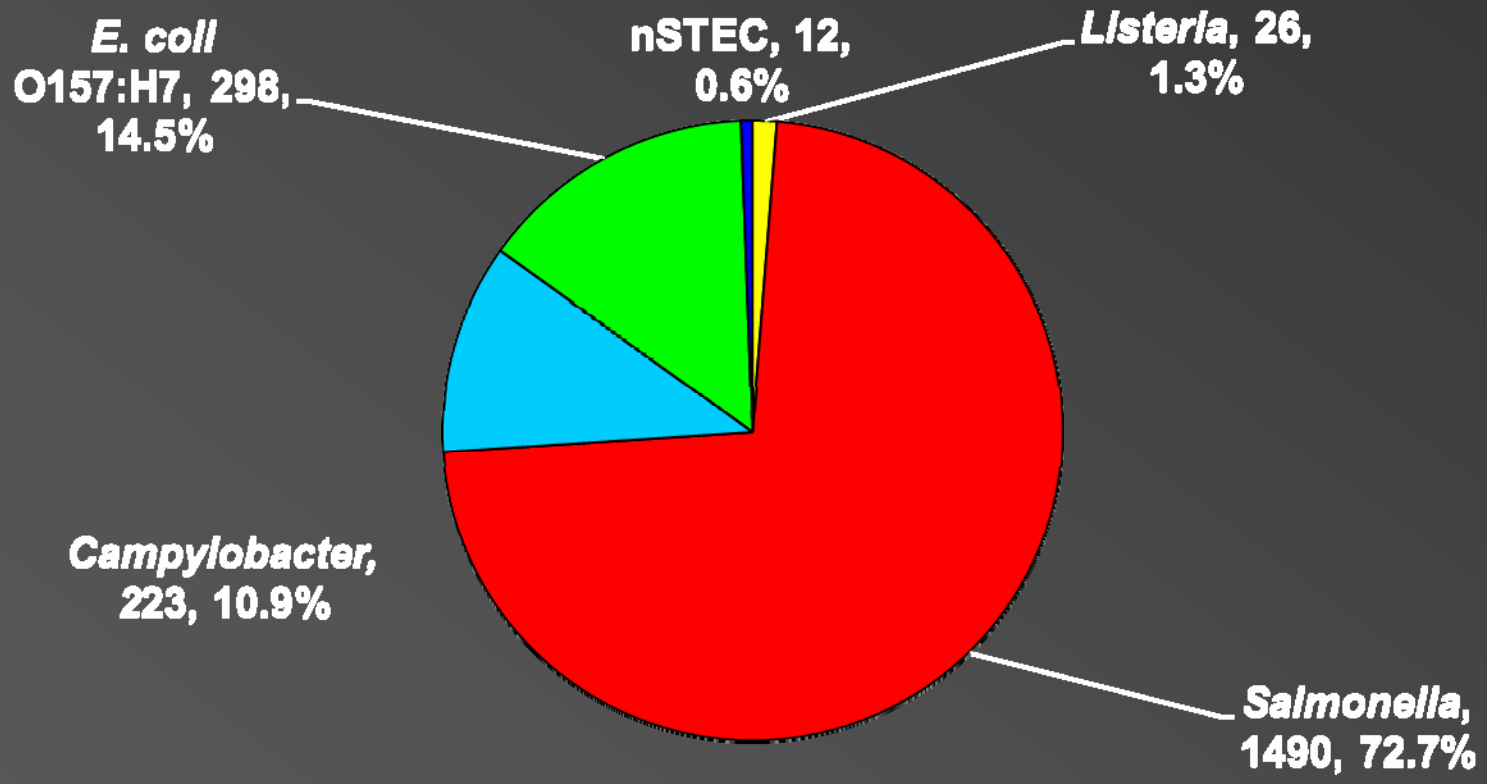
Source: Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M A, Roy SL, Jones JL, and Griffin PM. (2011). Foodborne illness acquired in the United States major pathogens. Emerg Infect Dis. 17(1): 7 15.

Foodborne Outbreaks: 1998-2007



Source: Outbreak Alert: Analyzing Foodborne Outbreaks 1998-2007, Closing the Gaps in Our Federal Food Safety Net, 2009. Center for Science in the Public Interest (http://cspinet.org/new/pdf/outbreakalertreport09.pdf).

CDC All Food Outbreaks Among Pathogens: 1998-2008



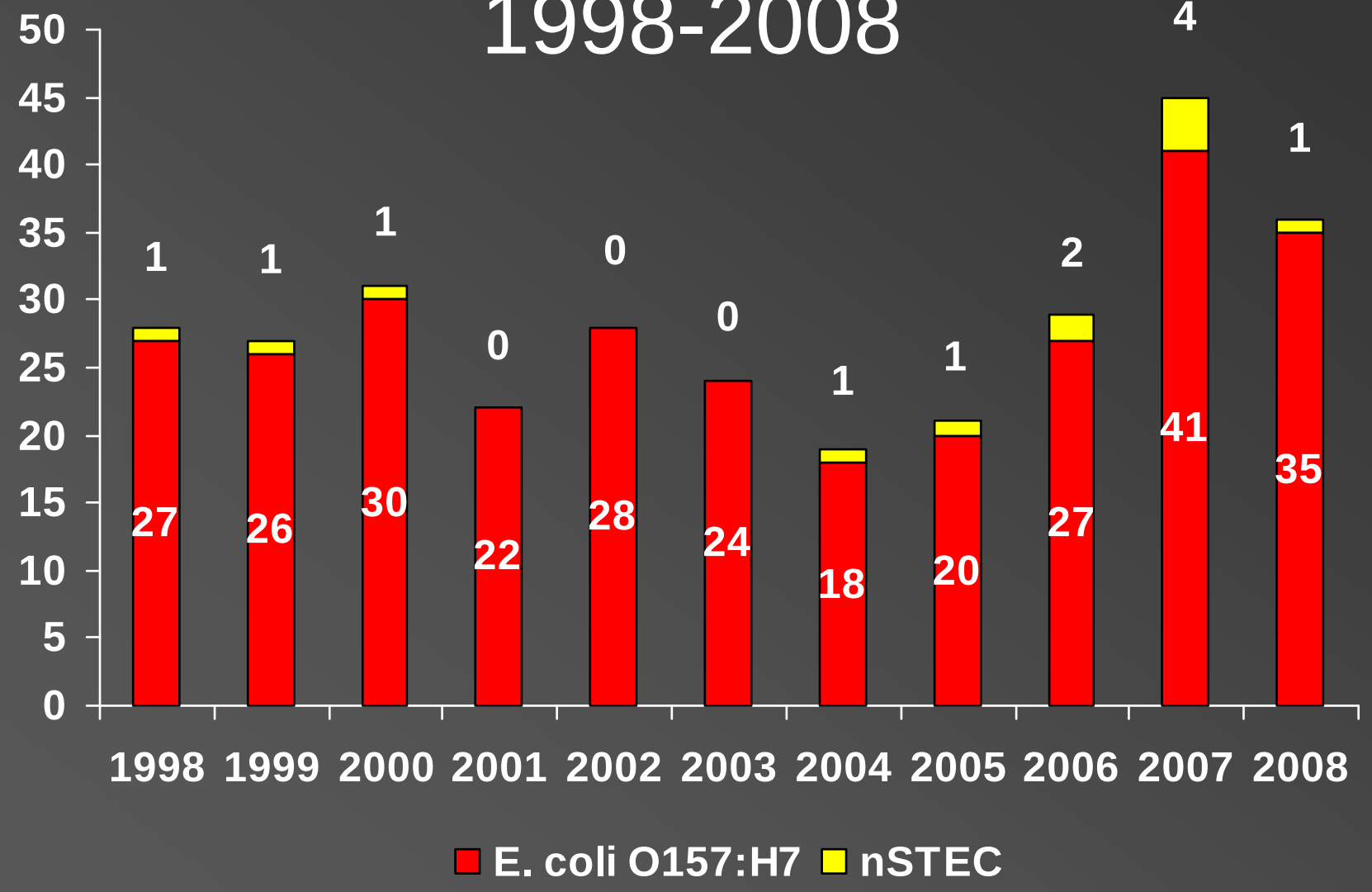
Source: CDC Foodborne Outbreak Online Database. http://wwwn.cdc.gov/foodborneoutbreaks/. Accessed February 18, 2011.

CDC Foodborne Outbreaks: 1998-2008

	O157	Non-O157
All Foods	298	12
Beef Related	93	0
% Beef Related	31%	0%

Source: CDC Foodborne Outbreak Online Database. http://wwwn.cdc.gov/foodborneoutbreaks/. Accessed February 18, 2011.

CDC All Food STEC Outbreaks: 1998-2008



Source: CDC Foodborne Outbreak Online Database. http://wwwn.cdc.gov/foodborneoutbreaks/. Accessed February 18, 2011.

Single Etiology Outbreaks

Serogroup	Number of Outbreaks
O111	7
O121	4
O26	3
O45	3
O145	1
O104	1
O103	1

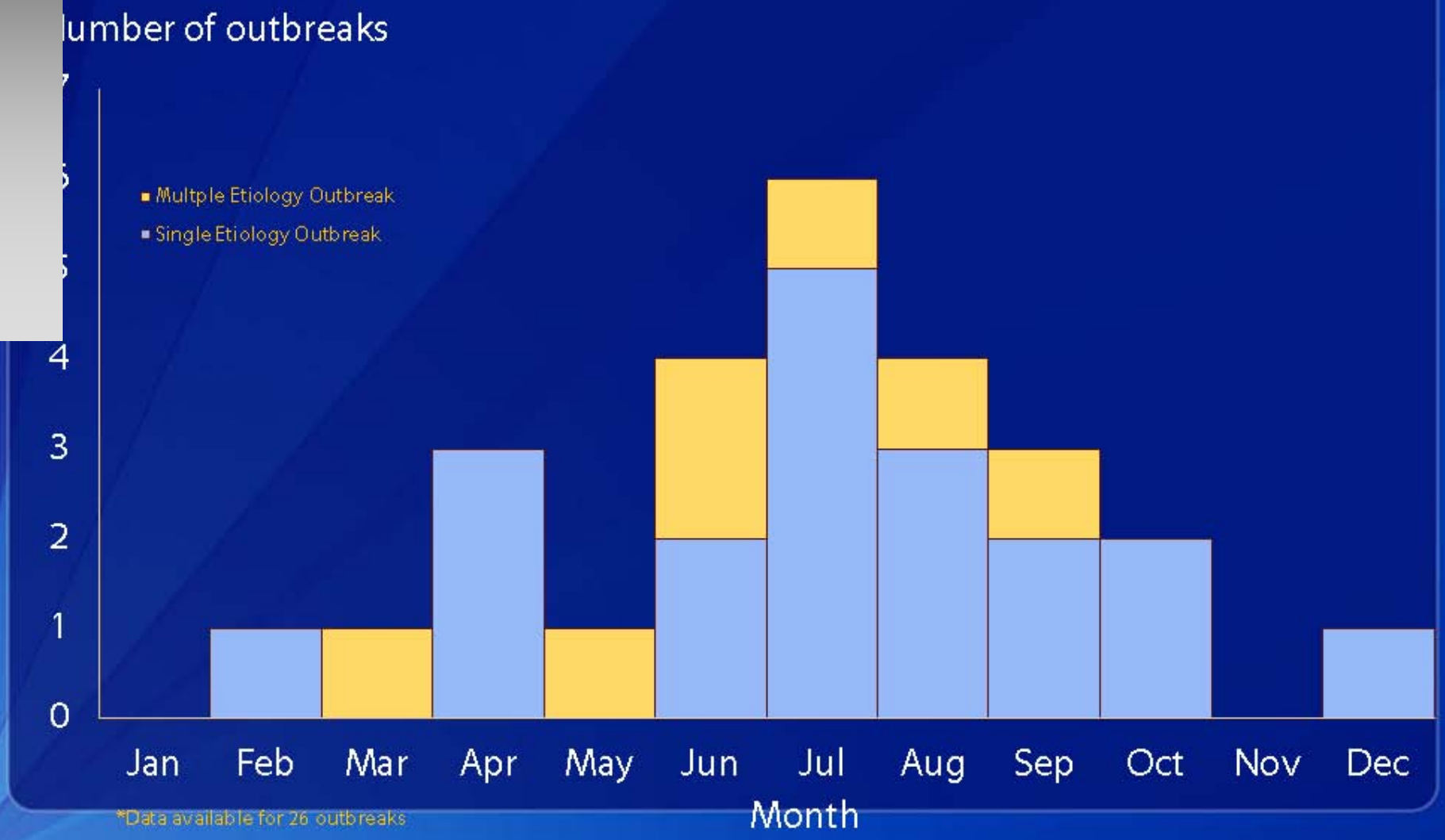
Source: Mody R and Luna RE. Surveillance for Non-O157 STEC Infections and Outbreaks, United States. CDC Enteric Disease Epidemiology Branch. Presentation. January 5, 2011.

Multiple Etiology Outbreaks

Serogroup (s)	Other Pathogens
O111	*Cryptosporidium*, *Campylobacter*, Salmonella
O111	*Campylobacter*, *E. coli* O157:H7
O111, O51 and O undefined	*Cryptosporidium*, *E. coli* O157:H7
O111	*Cryptosporidium*
O111	*Cryptosporidium*
O145	*E. coli* O157:H7, *Campylobacter*
O121, O26, and O84	

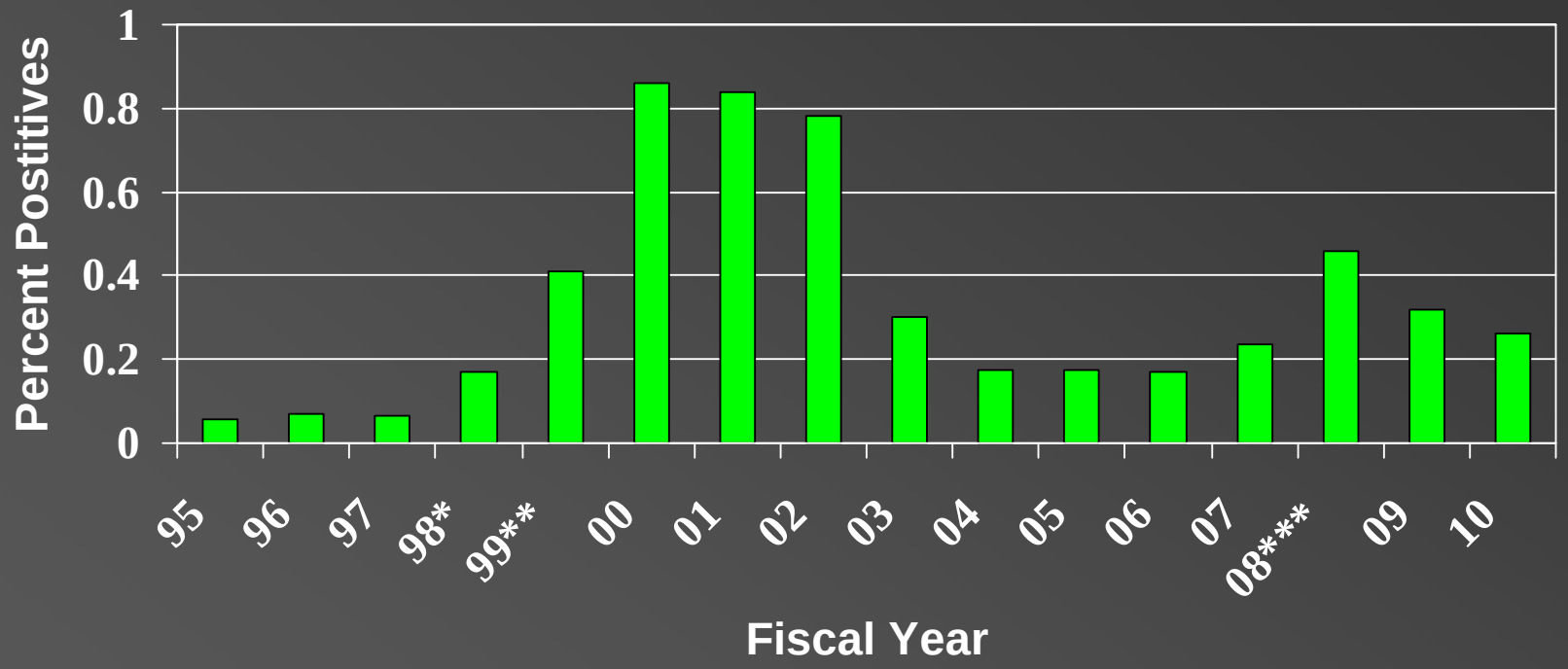
Source: Mody R and Luna RE. Surveillance for Non-O157 STEC Infections and Outbreaks, United States. CDC Enteric Disease Epidemiology Branch. Presentation. January 5, 2011.

Number of reported outbreaks of Non-O157 STEC Infection, 1990-2008, by Month*



Source: Mody R and Luna RE. Surveillance for Non-O157 STEC Infections and Outbreaks, United States. CDC Enteric Disease Epidemiology Branch. Presentation. January 5, 2011.

Prevalence of *E. coli* O157:H7 in Ground Beef[1]



[1] Results of individual raw ground beef products analyzed for *E. coli* O157:H7 in federal plants.
* In 1998 FSIS increased sample size from 25 g to 375 g.
** In July 1999 FSIS changed to a more sensitive analytical method.
*** In 2008, FSIS changed to a more sensitive enrichment broth
2010 data as of December 26

E. coli O157:H7 Recalls Meat Products

	2004	2005	2006	2007	2008	2009	2010	Total
No. of Recalls	6	5	8	20*	15	16	11***	81
No. of Recalls due to Illness Investigation (%)	3 (50%)	4 (80%)	0	10 (50%)	5 (33%)	5** (27%)	4 (36%)	31 (38%)
No. of Recalls due to FSIS/ Company Sample (%)	3 (50%)	1 (20%)	8 (100%)	10 (50%)	10 (67%)	11 (73%)	7 (64%)	50 (62%)

*Does not include August 30 Health Alert
**Recall associated with both a positive sample and illness outbreak is included in illness investigation
*** 2010 data does not include recall for *E. coli* O26

E. coli O157:H7 in Ground Beef

Calendar Year	2005[b]	2006	2007	2008[a]	2009
Unweighted Percent positive	0.16	0.17	0.23	0.45	0.30
Volume Weighted Percent Positive	0.50	0.47	0.31	0.37	0.26

[a] Beginning with CY 2008, annual microbiological sample results will be posted according to the date the sample was collected. Prior to CY 2008, yearly posting of microbiological data results was based on the sample analysis completion date. For this reason, data from CY 2008 can not be directly compared to CY 2007 and prior years. In addition to the change in date criterion, target sampling that incorporates production volume and results history was introduced as well as incorporating a change in the laboratory testing method.

[b] During October 2005, a new screening method was introduced to reduce the number of screen positives that do not confirm positive.

Source: Annual data from FSIS raw ground beef verification sampling program

STEC-
The Good, The Bad and The Ugly

Peter Gerner-Smidt
Centers for Disease Control & Prevention
Atlanta, GA, USA
plg5@cdc.gov

National Center for Emerging and Zoonotic Infectious Diseases

Division of Foodborne, Waterborne, and Environmental Diseases

Enteric Diseases Laboratory Branch

Peter Gerner-Smidt, M.D., D.M.S., Branch Chief
John Besser, PhD, Deputy Branch Chief
Karen Stamey, Branch manager
Nicole Rankine, QMS manager
4 FTE, 2 non-FTE

National Enteric Reference Laboratory Team
Patricia Fields, Ph.D.
12 FTE, 8 non-FTE

- ***Campylobacter* and *Helicobacter* Unit**
  Collette Fitzgerald, Ph.D.
- ***Escherichia* and *Shigella,* Unit**
  Nancy Strockbine, Ph.D.
- ***Salmonella* Unit**
  Patricia Fields, Ph.D.
- ***Listeria* ,*Yersinia* , *Vibrio,* and other *Enterobacteriaceae* Unit**
  Cheryl Tarr, Ph.D.

National Antimicrobial Resistance Surveillance Team
Jean Whichard, D.V.M., Ph.D.
4 FTE, 5 non-FTE

- **NARMS Surveillance Unit**
  Kevin Joyce
- **NARMS Applied Research Unit**
  Jean Whichard, D.V.M., Ph.D.

National Botulism Laboratory Preparedness Team
Susan Maslanka, Ph.D.
5 FTE, 3 non-FTE

- **Botulism Public Health Research Unit**
  Brian Raphael, Ph.D.
- **Botulism Outbreak Investigation Unit**
  Carolina Luquéz, Ph.D.

PulseNet USA Team
Efrain Ribot, Ph.D.
13 FTE, 7 non-FTE

- **PulseNet Database Unit**
  Kelley Hise, M.P.H.
- **PulseNet Methods Development Unit**
  Eija Hyytia-Trees, Ph.D.
- **PulseNet Reference Unit**
  Molly Freeman, Ph.D.

National Enteric Laboratory Diagnostics and Outbreak Team
Deborah Talkington, Ph.D.
7 FTE, 1 non-FTE

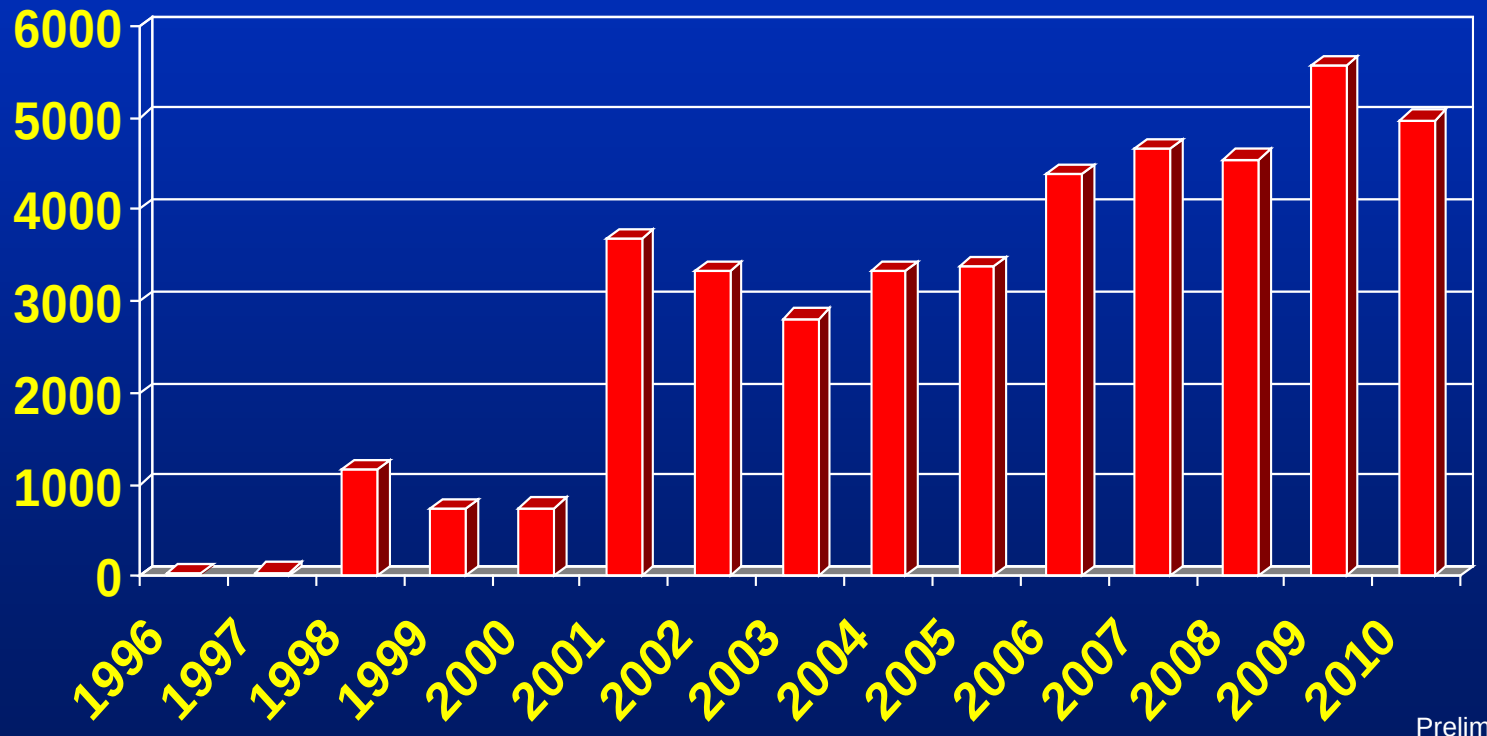
- **Epidemic Investigations Laboratory Unit**
  Cheryl Bopp, M.S.
- **Immunodiagnostics Unit**
  Deborah Talkington, Ph.D.

Role of EDLB in the Surveillance of STEC Disease

- ✓ Detect foodborne disease case clusters by PFGE
  - ➔ Facilitate early identification of common source outbreaks
- ✓ Assist epidemiologists in investigating outbreaks
  - ➔ Separate outbreak-associated cases from other sporadic cases (case definition)
  - ➔ Characterize and isolate STEC from clinical (and food) sources (strain identification)
  - ➔ Assist in rapidly identifying the source of outbreaks (culture confirmation)
  - ➔ Act as a rapid and effective means of communication between public health laboratories
- ✓ Research

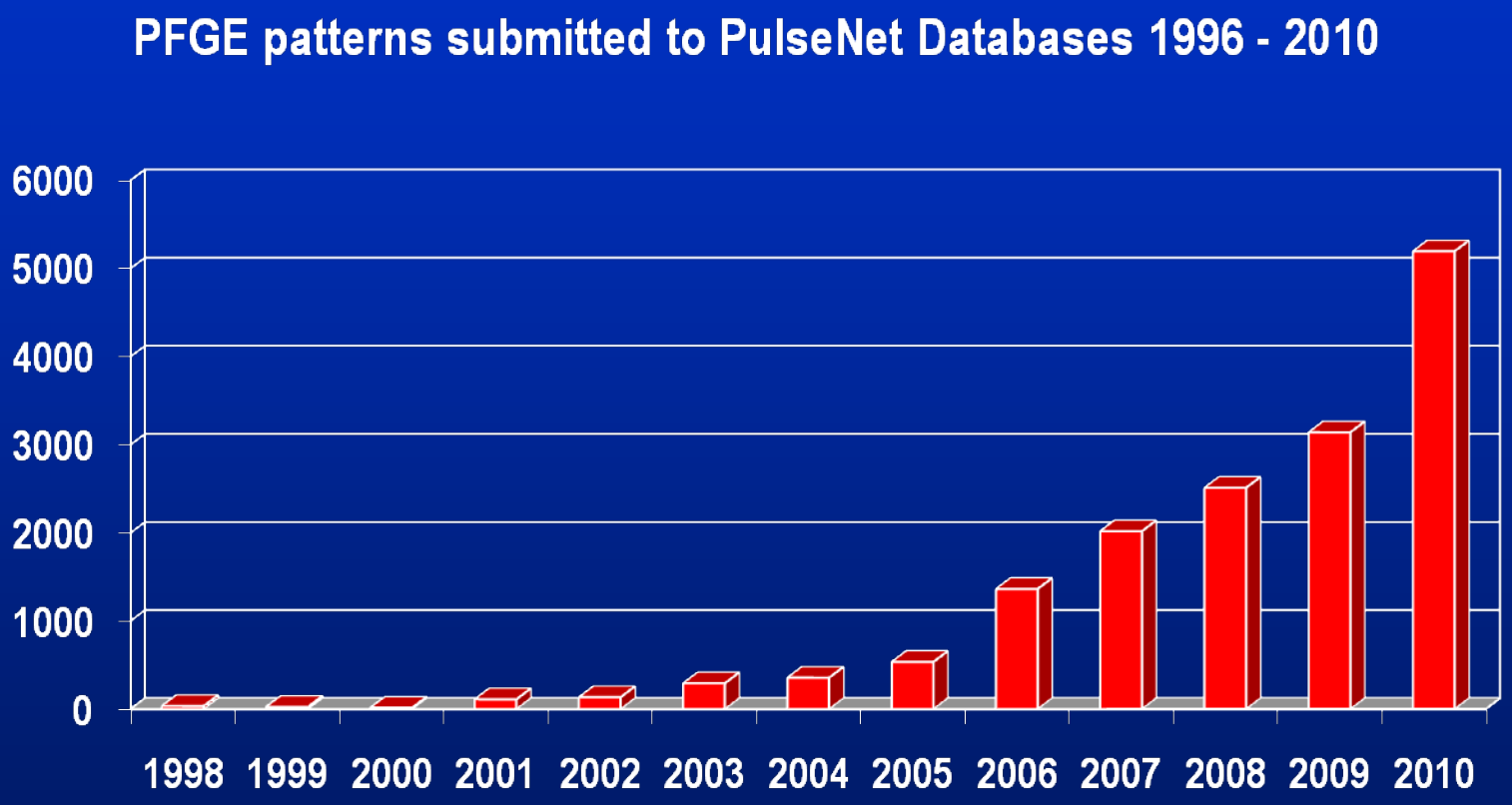
Annual Submissions of *Escherichia coli* O157 to PulseNet

PFGE patterns submitted to PulseNet Databases 1996- 2008



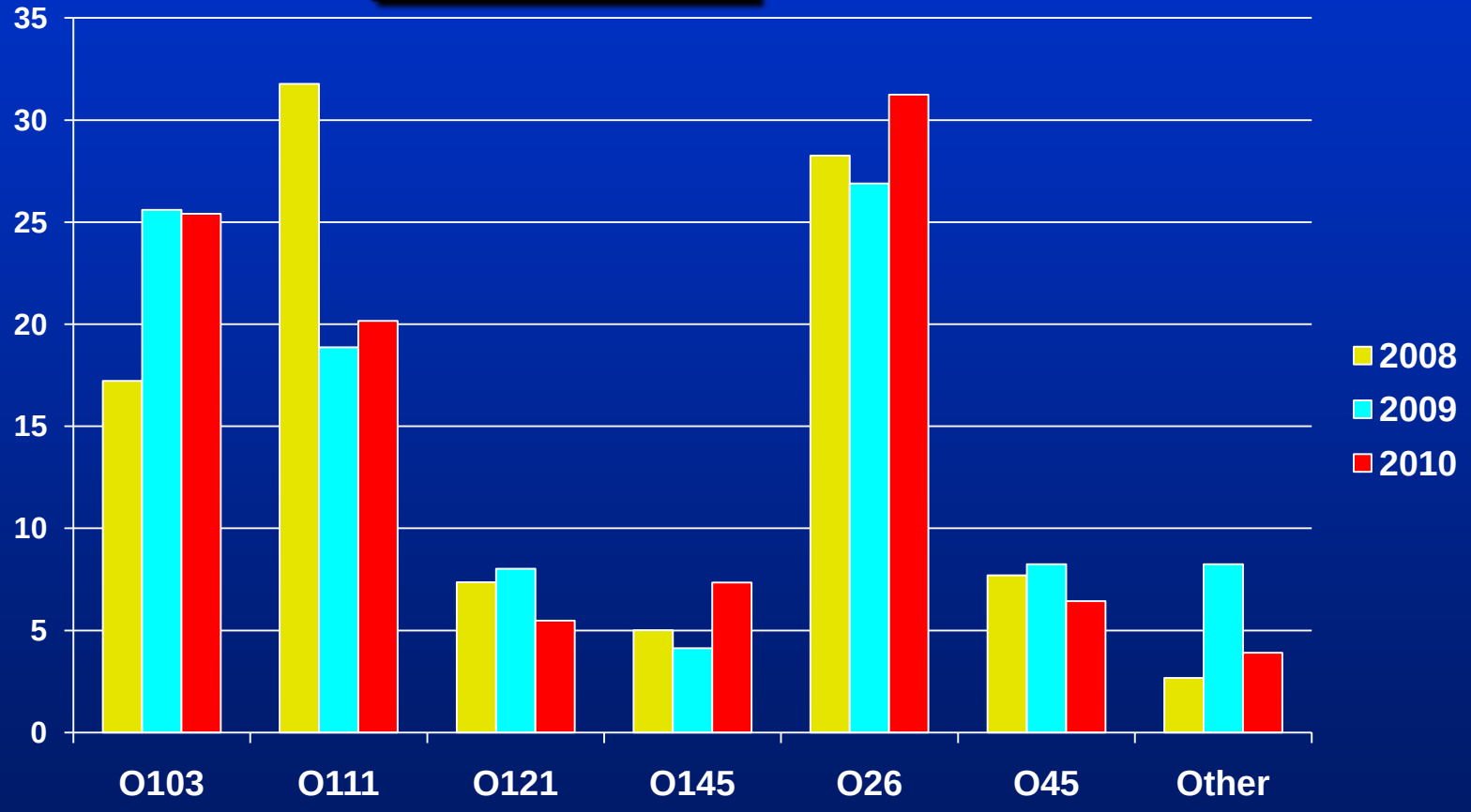
Preliminary data

Annual Submissions of non-O157 STEC to PulseNet



Preliminary data

% Distribution of Major Non-O157 STEC Serogroups Submitted to PulseNet 2008- 10



Preliminary data

CDC

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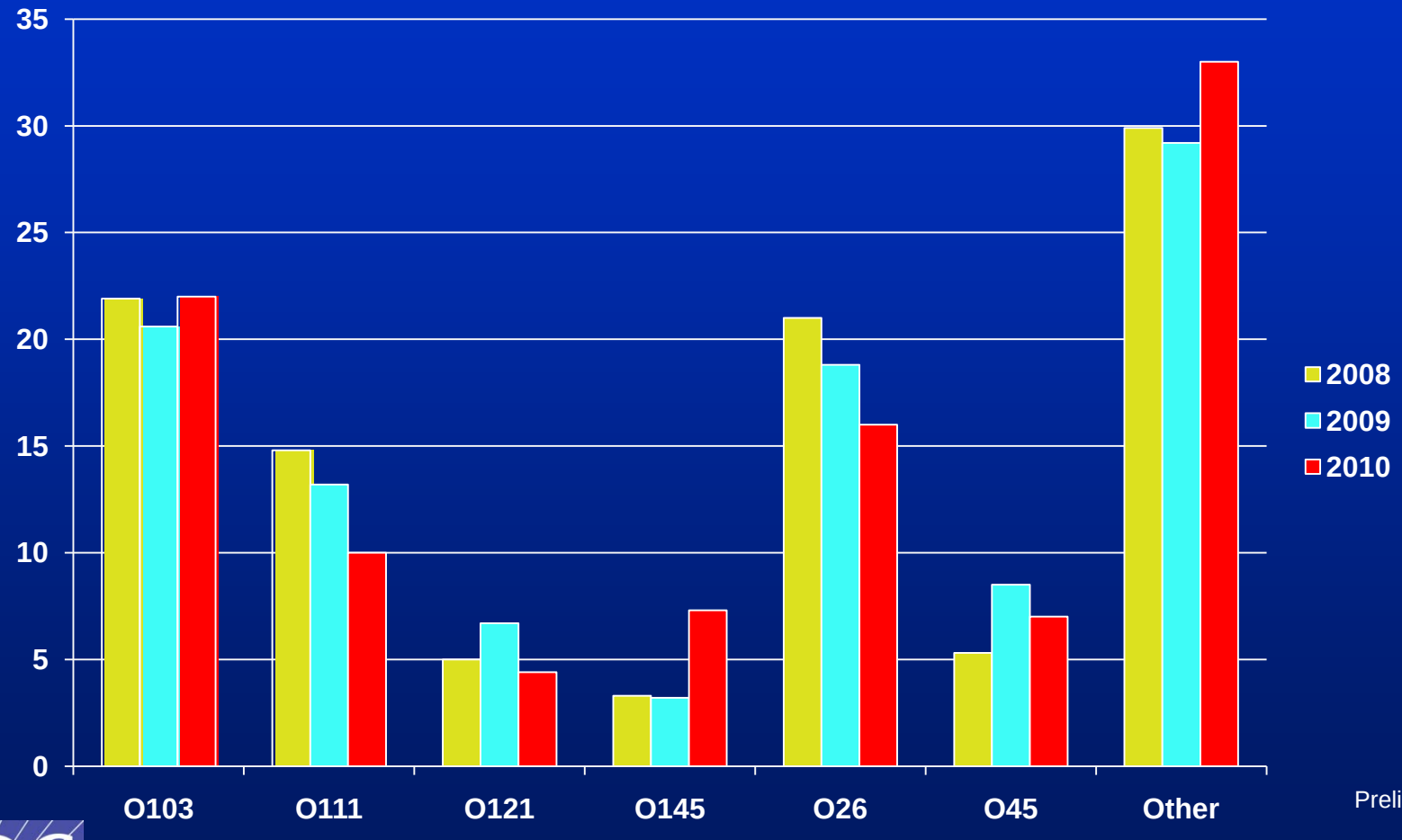
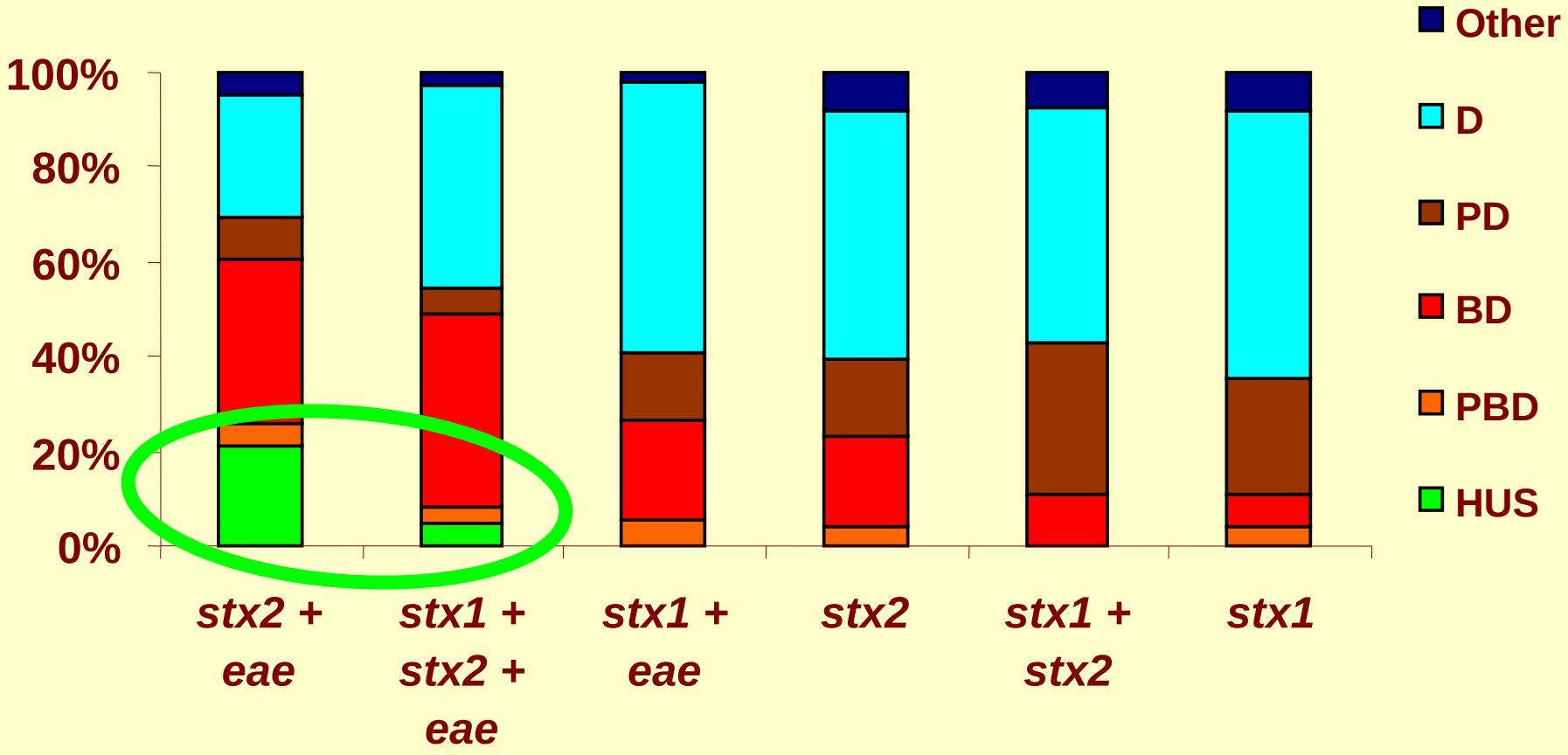
% Distribution of Major Non-O157 STEC Serogroups Submitted to CDC Reference Lab 2008-10 (N=2,344)



Preliminary data

CDC

SAFER • HEALTHIER • PEOPLE™

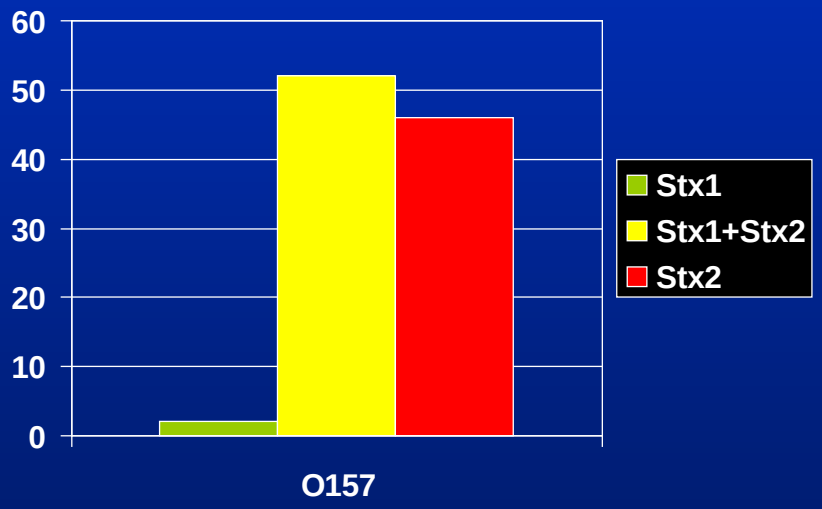


F. Scheutz USDA, FDA, CDC: Public non-O157 meeting, Washington DC 2007

The Serotype is NOT Independently Associated with Virulence

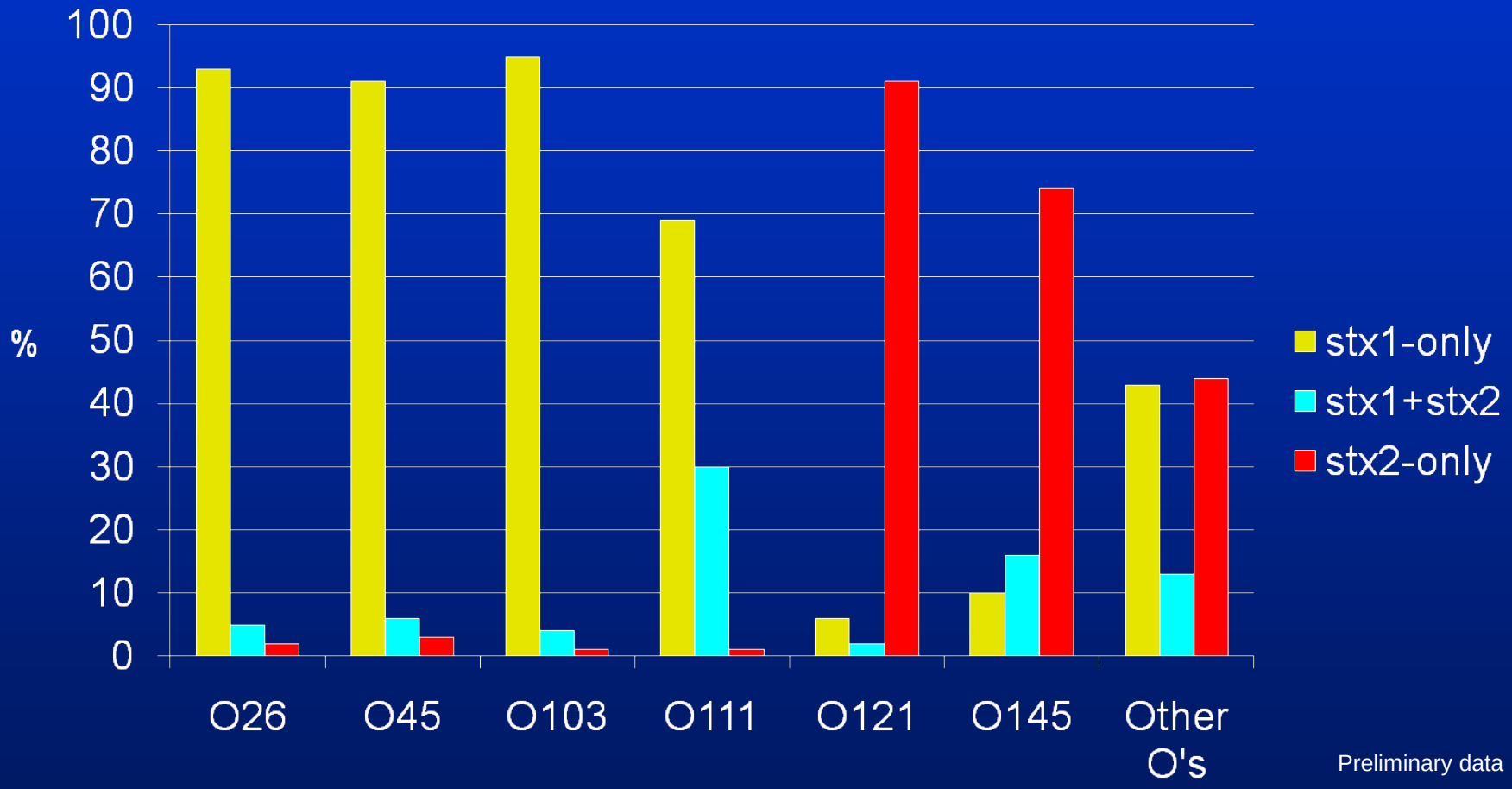
Ethelberg *et al.* Emerg Infect Dis 2004, 10: 842- 847

- O157 strains consistently contains more virulence determinants than most non-O157 serotypes



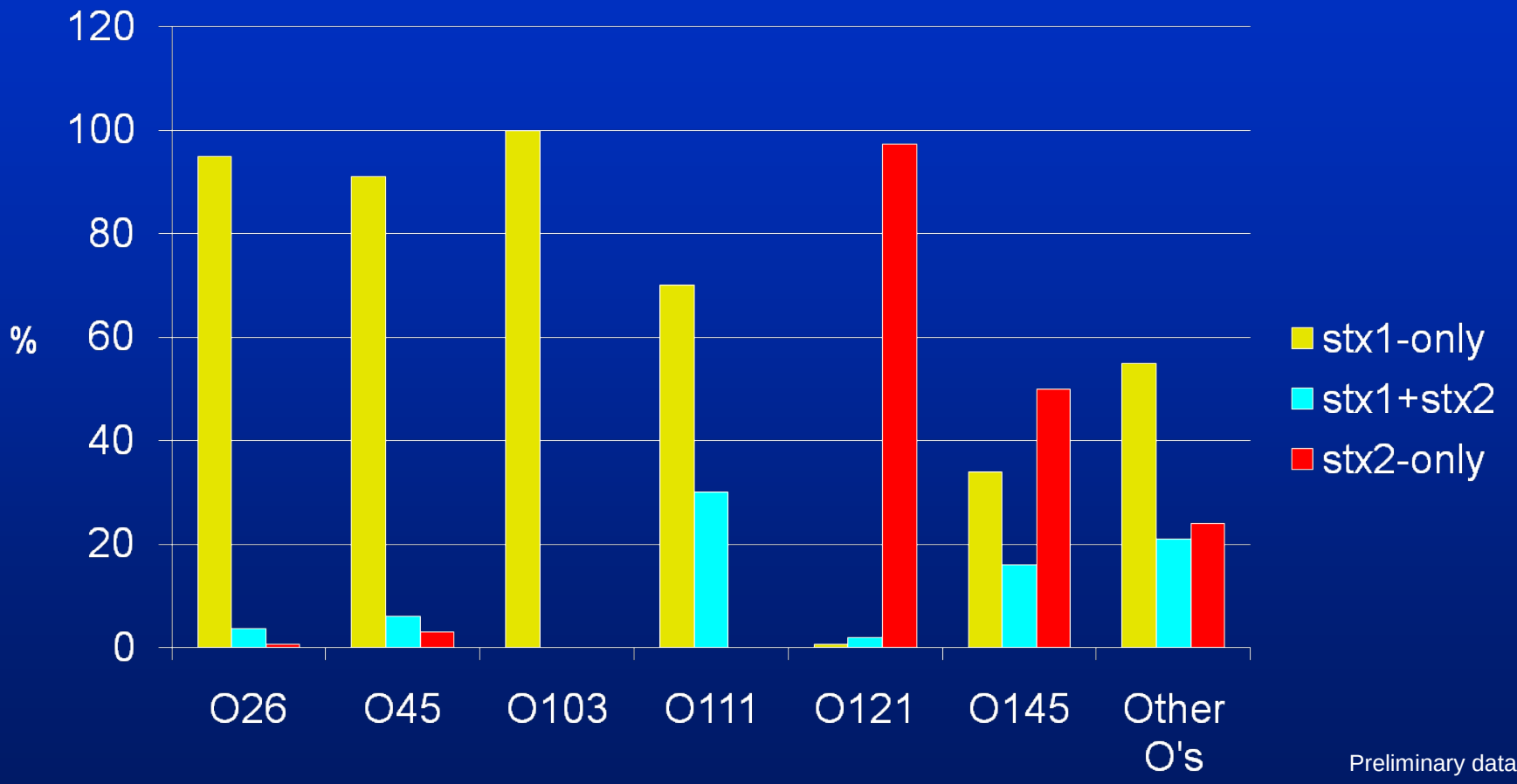
- Some strains belonging to a non-O157 serotype are as virulent as the most virulent O157 strains

Stx Profiles Among Top 6 Non-O157 STEC in United States submitted to PulseNet 2008- 2010 (N=980)



Preliminary data

Stx Profiles Among Top 6 Non-O157 STEC in United States submitted to CDC Reference Lab 2008- 2010 (N=2,344)



Preliminary data

HUS in non-O157 STEC in the United States

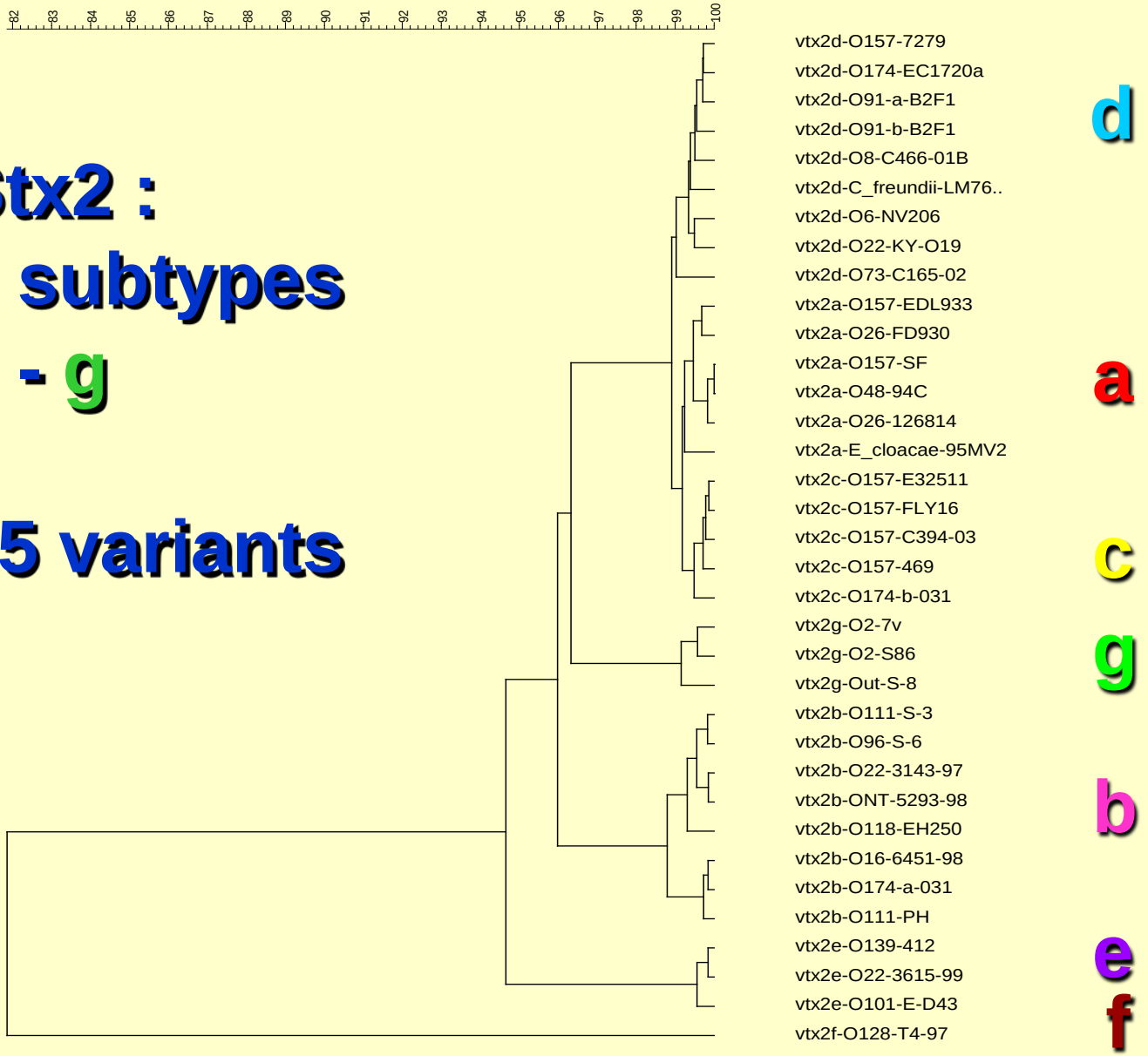
O-Group	Stx_1 - only	Stx_2 - only	Stx_1 + Stx_2
O111	1/65	-	10/87
Other	0/505	8/200	3/82
Total	1/570 (0.2%)	8/200 (4%)	13/169 (8%)

Brooks *et al.* JID 2005; 192:1422- 1429

Pairwise (OG:100%,UG:0%) (FAST:2,10) Gapcost:0% Disc. unk.
vtx_TRANSL

Stx2 :
7 subtypes
a - g

35 variants



F. Scheutz USDA, FDA, CDC: Public non-O157 meeting, Washington DC 2007

Attack rate of Stx2 subtypes associated with HUS (DK)

O157		
Stx2a + Stx2c	7/24	29%
Stx2a	3/17	18%
Stx2c	1/18	6%

Non-O157		
Stx2a	6/20	30%
Stx1b + Stx2a	2/8	25%
Stx2a + Stx2c	1/2	50%

STEC and HUS in Recent Outbreaks

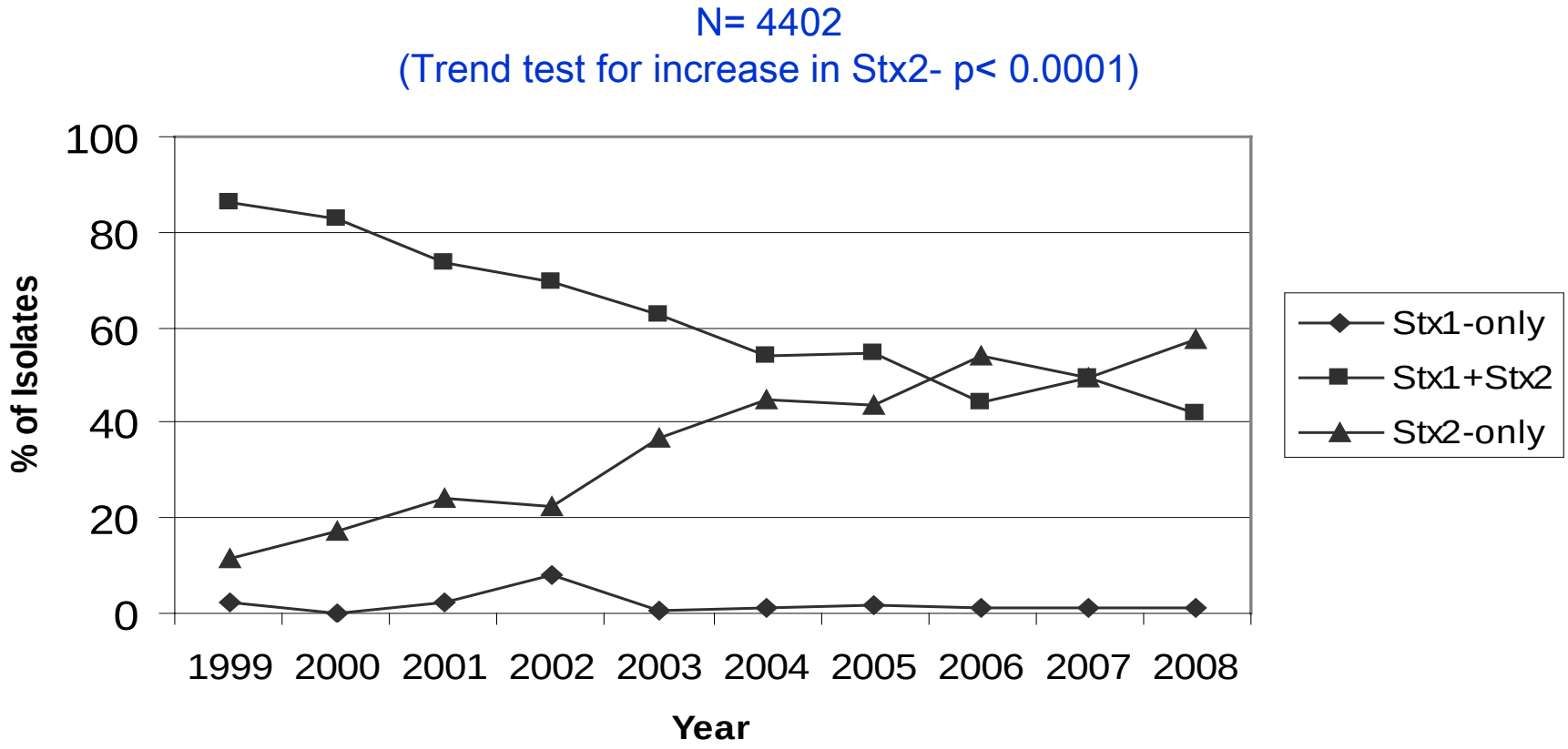
- Norway 2006 (fermented sausage) - **O103:H25**
  - 17 case patients
  - 10 HUS (**59%**)
  - *stx2* (subtype *stx2a*)
    Schimmer *et al*. 2008 BMC Infect Dis.8(1): 41
- United States 2006 (spinach) - **O157:H7**
  - 199 case patients
  - 31 HUS (**16%**)
  - *stx2* (subtypes *stx2a* + *stx2c*)
    CDC 2006 MMWR 55(38);1045-1046
- 2008 (Restaurant) – **O111:NM**
  - 341 case patients
  - 25 HUS (7%)
  - Stx1+Stx2
    http://www.ok.gov/health/documents/EcoliO111SummaryReport.pdf

HUS in Recent STEC Outbreaks in the United States

- 2009 (Cookie Dough) - **O157:H7**
  - 76 case patients
  - 10 HUS (**13%**)
  - Stx2
- 2009 (Ground Beef) - **O157:H7**
  - 23 case patients
  - 2 HUS (**9%**)
  - Stx1 + Stx2
    http://www.cdc.gov/ecoli/2009/
- 2010 (Romaine lettuce) – O145:NM
  - 30 case patients
  - 3 HUS (10%)
  - Stx2a

Preliminary data

Stx Types in Human O157 Isolates Submitted to PulseNet 1999- 2008



M. Leeper, Thesis 2009

Most common PFGE patterns of STEC O157 in ground beef 2001- 2006

pattern name	isolate count	GB rank	human rank
EXHX01.0074	9	1	2
EXHX01.0047	8	2	1
EXHX01.0008	5	3	11
EXHX01.0224	4	4	3
EXHX01.0800	3	5	none*
EXHX01.0124	3	6	4
EXHX01.1343	3	7	5
EXHX01.0011	3	8	6
EXHX01.1058	2	9	none*
EXHX01.2178	2	10	none*
EXHX01.0200	2	11	9
EXHX01.0097	2	12	12
EXHX01.0238	2	13	26
EXHX01.0272	2	14	28
EXHX01.1401	2	15	120
EXHX01.1068	2	16	low, 3 cases
EXHX01.1354	2	17	low, 1 case

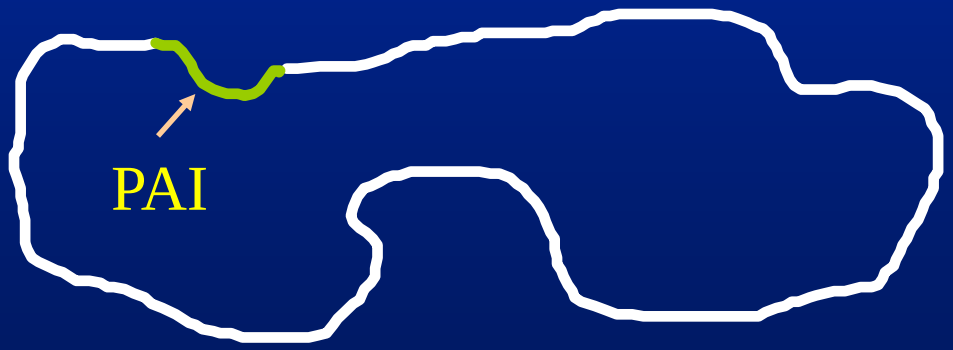
W. Lanier, Thesis 2008

Virulence Factors in STEC

Virulence factor	Gene Location
Shiga toxin (*stx*)	Phage
Intimin (*eae*)	PAI (LEE)
Enterohemolysin (EhxA, HlyA)	Plasmid (pO157)
Non-LEE effectors (*nle*)	PAI's
Saa adhesin (STEC autoagglutinating adhesin)	Plasmid
Subtilase	Plasmid
More……	

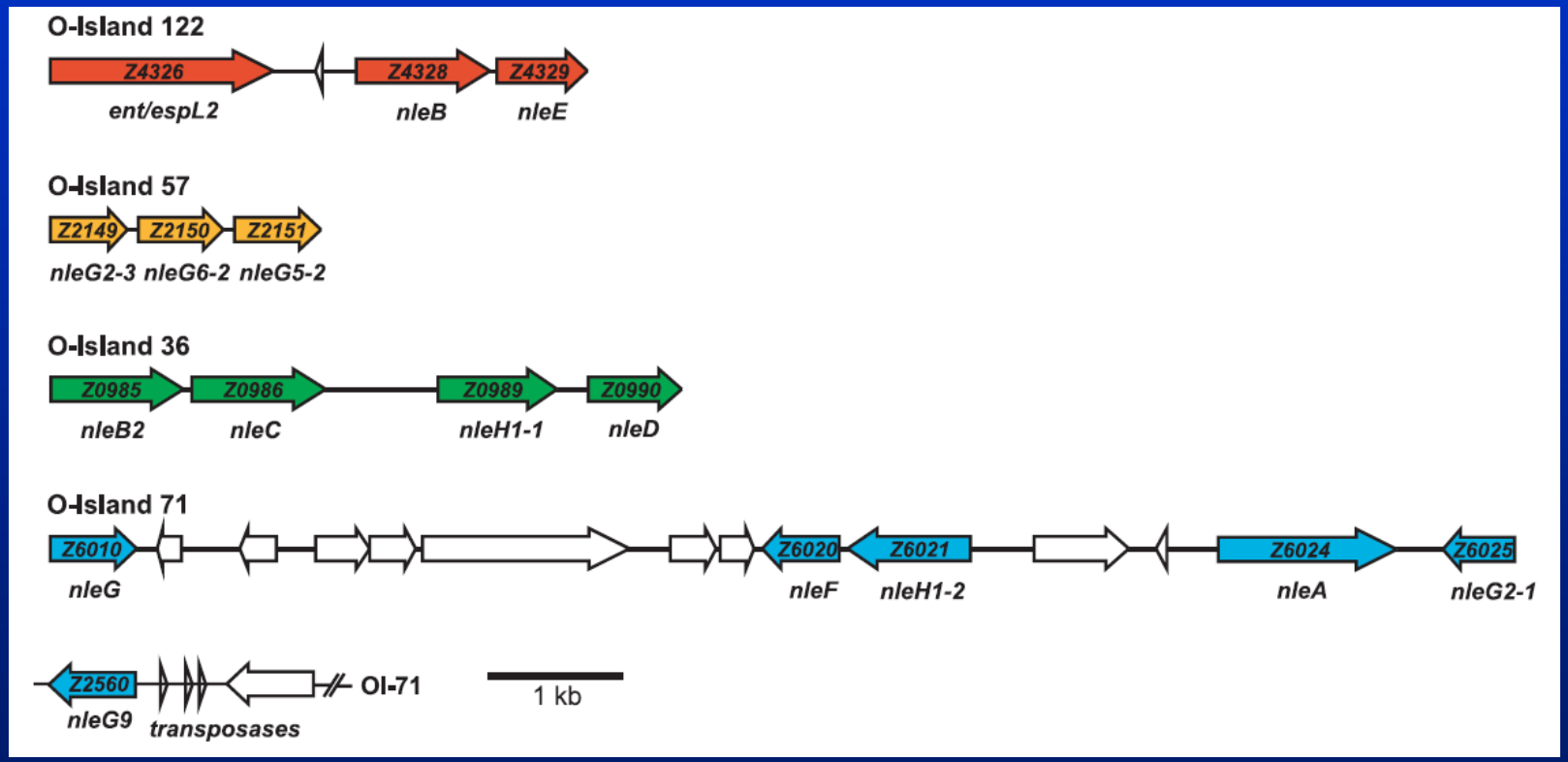
Locus for Enterocyte Effacement (LEE)

- Pathogenicity island (PAI) in EPEC and STEC
- Contains genes encoding intimin *(eae),* intiminreceptor (*tir*), type III secretion system, regulators
- Adherence to the enteric epithelium and attaching and effacing enteric lesions



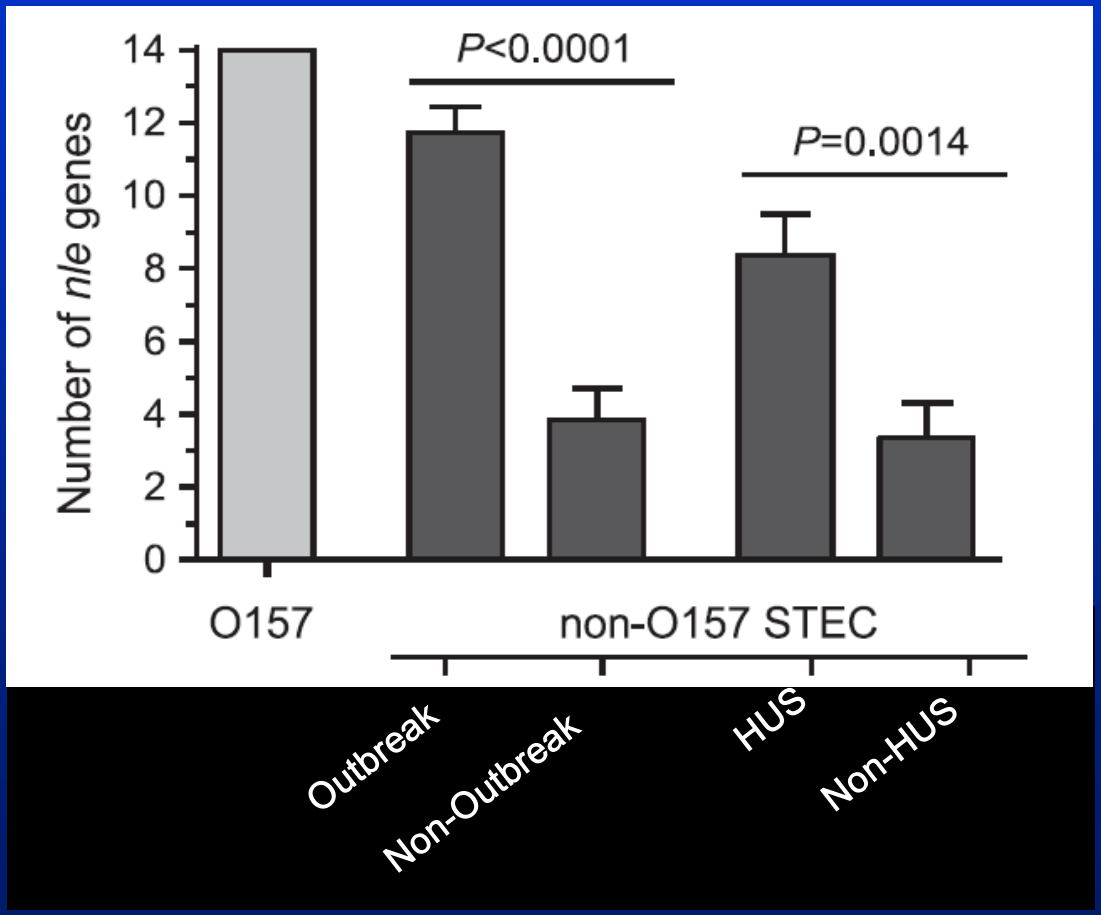
Non-LEE effectors (*nle*)

- At least 16 *nle* genes in at least four PAI's



Karmali *et al.* JCM 2003, 41: 4930- 40; Coombes *et al.* AEM 2008, 74: 2153-60

Non-LEE effectors (*nle*) and Disease



Wickham *et al.* 2006; 194:819–27

New Paradigm

Classification of STEC in **5 Sero-Pathotypes**

Karmali *et al.* , 2003, J. Clin. Microbiol., 41:4930-40

Based on the reported occurrence of serotypes in human disease, in outbreaks and/or in hemolytic-uremic syndrome (HUS)

Sero-pathotype	Relative incidence	Frequency of involvement in outbreaks	Association with severe disease (HUS or HC)	Serotypes
A	High	**Common**	**Yes**	**O157:H7, O157:NM**
B	Moderate	**Uncommon**	**Yes**	**O26:H11, (O45), O103:H2, O111:NM, O121:H19, O145:NM**
C	Low	Rare	Yes	**O91:H21, O113: H21, O104:H21, others**
D	Low	Rare	No	**multiple**
E	Non- human only			**multiple**

New Paradigm

Problems with the sero-pathotype classification

- **Association with serotype and not with virulence profile**
  - O157 is virulent because it almost invariably contains Stx2a and or Stx2c
  - More than 120 O:H serotypes have been associated with HUS (Bergey's Manual of Systematic Bacteriology, 2nd ed.)
  - Many O:H serotypes display extensive heterogeneity
- **Involvement in outbreak may rapidly change**
- **But the top 6 non-O157 serotypes in the US has not changed the past 20 years**

Modified from F. Scheutz USDA, FDA, CDC: Public non-O157 meeting, Washington DC 2007

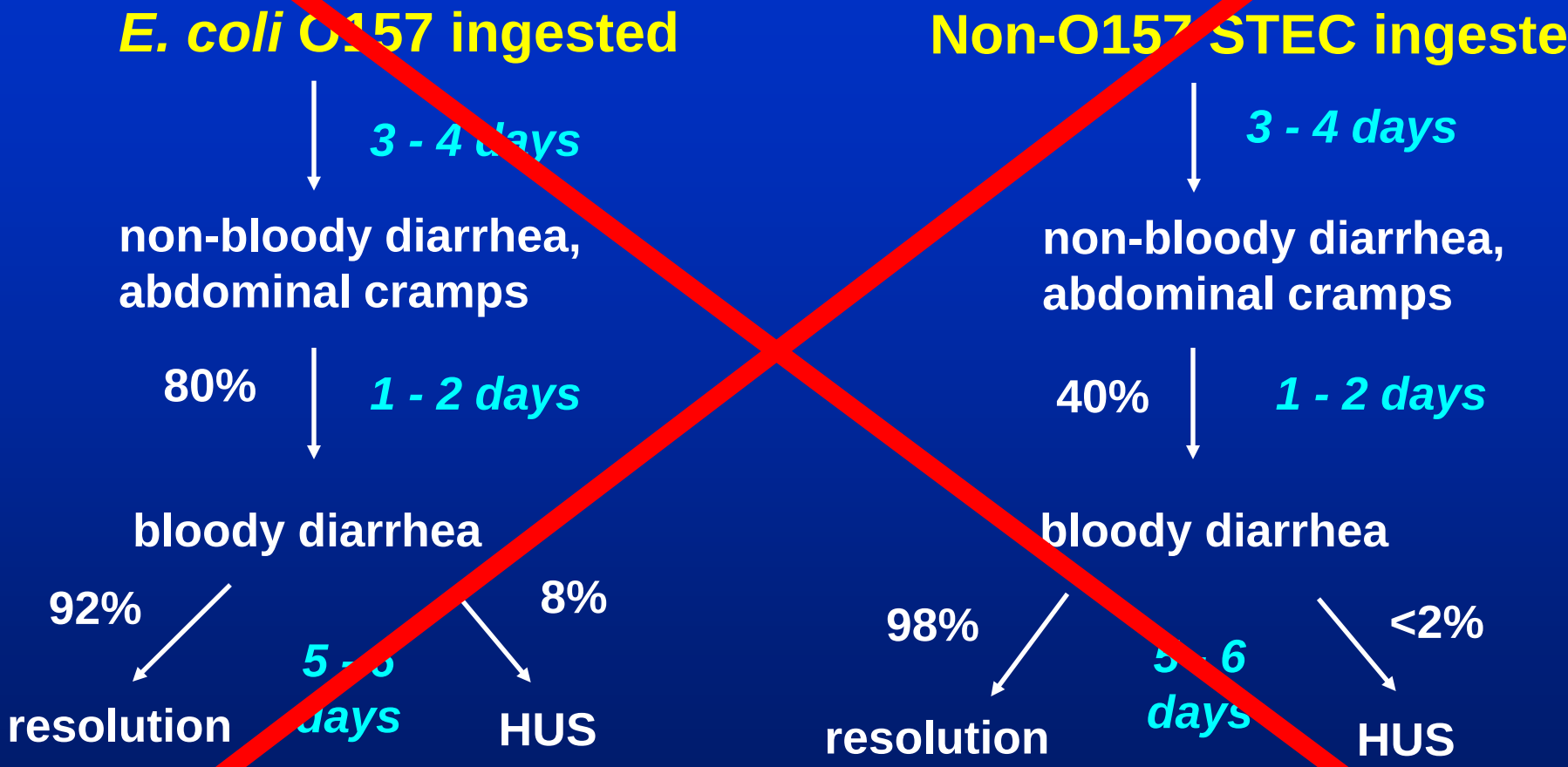
Alternative Classification

To be developed

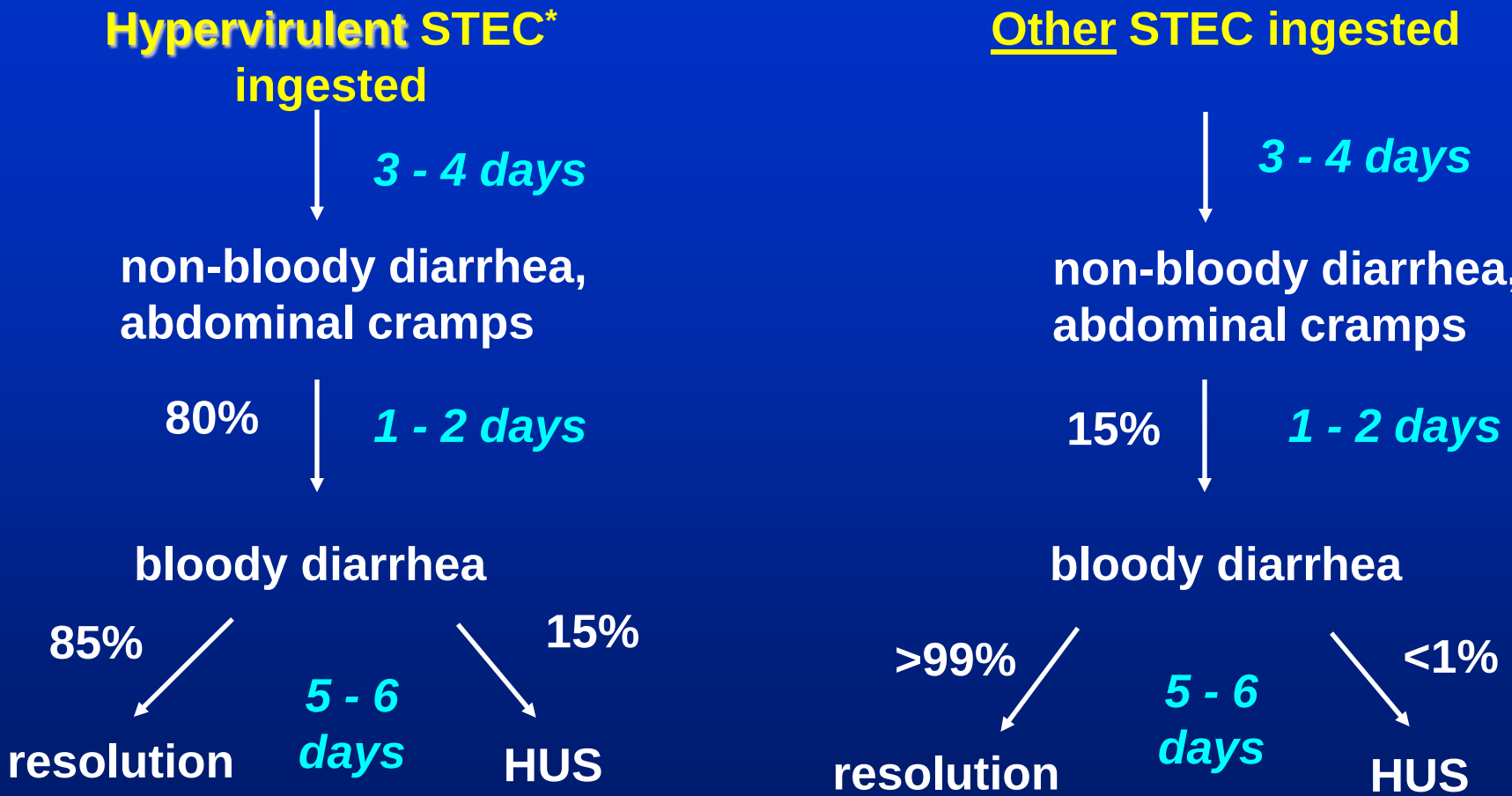
- **Pathogenecity index (PI)**
  - Stx profile
  - *eae*
  - Other virulence genes/factors (e.g. *nle, saa,* subtilase)

1. Severe disease – PI high
2. Diarrhea in humans – PI intermediate
3. Animal STEC's – PI low

Sequence of events in STEC infection



Sequence of events in STEC infection



* Hypervirulent STEC:
- Stx2a and/or Stx2c, eae, many nle
- Stx2d and NO eae, many nle

STEC Characterization and Diagnostics in the 21st Century

DNA array:

1. Virulence Profile
2. Molecular Serotype
3. SNP-Type

e.g. *stx1*, *eae*, *nleA*, *nleB*, O26, H2, A-G-C-A-A-T-G-C-C-C

- Virulence Information - Clinician
- Cluster Detection – Public Health
- Follow Trends – Public Health

STEC Characterization and Diagnostics in the 21st Century

DNA array:

1. Virulence Profile
2. Molecular Serotype
3. SNP-Type

- CDC is working on such an assay to characterize STEC isolates
- The technology to adapt the assay to the diagnostic setting is currently NOT available

Comments and Conclusions (1)

- STEC is a very heterogenous group of bacteria
  - Animals are the primary reservoir for probably All STEC
  - Some are human pathogens
  - Some are not human pathogens
- O157 is the most virulent serogroup
  - Some strains are likely not very virulent
- Some non-O157 strains are just as virulent as O157

Comments and Conclusions (2)

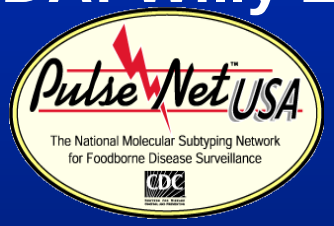
- The diagnostics approach to pathogenic STEC differ between human clinical samples and food/veterinary samples
  - The presence of STEC is diagnostic in humans
  - More detailed characterization is needed for non-human samples since not all STEC's are pathogens
  - A rapid diagnostic test that can provide a detailed characterization of STEC would be useful for any sample, but we are years away from that goal

Comments and Conclusions (3)

- Currently, an assay that will diagnose O157 and the top 6 non-O157 serogroups in food and animals seems to be the only feasible approach to capture most human pathogenic STEC isolates

Acknowledgements

- CDC: Nancy Strockbine, Cheryl Tarr, Cheryl Bopp, Kathy Greene, Molly Leeper, Hannah Gould, Patricia Griffin
- Statens Serum Institut: Flemming Scheutz
- USDA: Willy Lanier



Disclaimer:

"The findings and conclusions in this presentation have not been formally disseminated by the Centers for Disease Control and Prevention and should not be construed to represent any agency determination or policy."

Questions

1. What is the cost of performing the FSIS STEC test as outlined in MLG 5B?
   - How does it correspond with current *E. coli* O157:H7 testing?
2. How will the testing for these microorganisms outlined in MLG 5B increase the volume of samples compared to current *E. coli* O157:H7 testing?
3. How does the addition of testing for these microorganisms outlined in MLG 5B impact the inspector within the establishment?
   - Is there an increase labor time and associated costs?
   - Can the current infrastructure for federal inspectors at the establishment support the increased testing and analysis of these organisms?
   - Does this impact the turn around time of testing samples to the laboratory?
   - How does that impact the final time needed to get confirmed results?
4. How does the addition of testing for these microorganisms outlined in MLG 5B impact the labor needs and associated expense for laboratory personnel?
   - If so, what are the estimates in times and costs associated?
   - Can the current infrastructure in the laboratory support the increased testing and analysis of these organisms?
   - Does that impact the final time needed to get confirmed results?
   - What impact does that have on holding product within the facility?
   - What is the estimated percentage of product being held awaiting test results and how does it compare with current *E. coli* O157:H7 testing?
   - Are there any estimates of costs on holding product?
   - Does it correspond with current *E. coli* O157:H7 testing?
5. What is the cost of performing the FSIS STEC test as outlined in MLG 5B, if multiple "O" groups are positively identified?
   - Will that increase testing time?
   - Will it delay testing results to the inspector and/or the establishment?
   - How does that affect inspector time, labor, *etc.* (See Questions 2 & 3)?
   - What impact does that have on holding product within the facility?
   - What is the estimated percentage of product being held awaiting test results and how does it compare with current *E. coli* O157:H7 testing?
   - Are there any estimates of costs on holding product?
   - How does it correspond with current *E. coli* O157:H7 testing?
6. How will the testing for these microorganisms outlined in MLG 5B increase the volume of samples compared to current *E. coli* O157:H7 testing, if multiple "O" groups are positively identified?
7. What is the false positive rate of the FSIS STEC test as outlined in MLG 5B?
8. What is the false negative rate of the FSIS STEC test as outlined in MLG 5B?
9. Has the FSIS STEC test as outlined in MLG 5B been validated?
   - If so, by whom?
   - If not, why?
10. Are there any commercial test kits or methodologies available for the meat and poultry industry to use that meet current expectations in testing time, sensitivity, *etc*. compared to current *E. coli* O157:H7 products?
    - Are these methods/kits been validated?
    - If not, why?
    - Has FSIS equivalency requirements for these commercial tests been established?

    a. How can this occur if FSIS is still working finalizing their methodology?
11. Is the FSIS method as outlined in MLG 5B finished?
    - Can all microorganisms be tested for?
    - Have all the immunomagnetic beads been developed for the microorganisms?
        a. If not, what is the delay and when/how will the situation be rectified?
    - Are they sensitive for fitness of use as expected by FSIS?
    - Are there issues with enrichment media?
    - Are there issues with other media?
    - Can the FSIS method as outlined in MLG 5B be run on non-spiked samples and still be as sensitive and accurate as expected by FSIS?
12. Does FSIS have an estimate of the U.S. prevalence of the microorganisms outlined in MLG 5B in the raw ground beef components population?
    - Is this based on U.S. data?
    - If not, why as comparison of other countries prevalence numbers is not accurate based on differences in microorganism environmental ecology and inspection systems?
    - Is that data publicly available?
    - If not, why?
13. If the current sampling program (N=60) is changed to include more samples, how does that impact the above questions on FSIS regulated product?
    - Product purchased through AMS?
14. What is the public health risk of the microorganisms outlined in MLG 5B in the raw ground beef components population?
    - Has a risk assessment been performed utilizing U.S. data?
    - If not, why?
    - What data is needed to perform such a risk assessment?
15. What impact does testing for the microorganisms outlined in MLG 5B have on U.S. international trade policy?
    - Does it impact importation of beef products?
    - Has that been considered?
    - If not, why?