



Non-O157 Shiga Toxin Producing *Escherichia coli* (STEC) Workshop

Meeting Summary Beef Safety Research

OVERVIEW

Invited participants from international and domestic academic institutions, the meat industry, meat industry trade associations, and regulatory agencies, gathered at the Gaylord National Hotel and Convention Center in Washington D.C., on May 25-26, 2011 for a meeting on Shiga toxin-producing *Escherichia coli* (STEC). The meeting addressed STEC belonging to serotypes other than O157 (non-O157 STEC) as an emerging public health concern. Sessions were designed to review the current state of the science on the target organisms; traditional and rapid methodology for testing food samples; and global perspectives on testing, surveillance, risk characterization, and virulence; plus breakout sessions to address key questions and identify research gaps. With invited speakers and expert panel perspectives, participants gained critical knowledge about the target organisms and the limitations that must be overcome to globally address these organisms and protect public health.

SESSION 1: OVERVIEW OF STEC SCIENCE

Pina Fratamico, Ph.D., USDA-ARS, co-chair

Mohammad Koohmaraie, Ph.D. IEH Laboratories, co-chair

During the opening session, four invited speakers provided key information on pathogenicity, virulence factors, and prevalence, as well as international perspectives on the target organisms.

Invited speakers included: Brian Coombes, Ph.D., McMaster University, Canada; Denise Eblen, Ph.D., USDA-FSIS; Declan Bolton, Ph.D., Teagasc, Ashtown Food Research Center, Ireland; and Roger Johnson, Ph.D., Public Health Agency of Canada.

Key Points:

- Pathogenicity can be defined as the ability of an organism to induce moderate to severe illness in a host, done by using virulence factors. Virulence factors are genetically-encoded microbial molecules that, if present in proper combinations, could cause illness.
- Most virulence factors are acquired on mobile genetic elements, thus providing a means for continual evolution of the target organisms and making STEC diagnostics a challenge. Adherence to epithelial cells requires a Type III Secretion System (T3SS). The system also allows for attachment to other surfaces, including produce and other foods.
- STEC seropathotype grouping was introduced based on a 5-point scale (A through E) (A = associated with outbreaks, HUS, hemorrhagic colitis, and as an example, contains the serotype O157:H7, where E = no human infections reported and consists of over 40 serotypes).
- In the European perspective, prevalence of pathogenic *E. coli* (referred to as verotoxigenic *E. coli* or VTEC in Europe and Canada) in 2009 was reported at 2.3% in beef and O157 at 0.7%. The organisms are considered ubiquitous in cattle populations and seasonal peaks are observed as similar to the peaks seen in the U.S. with O157. Though one study mentioned serogroups O113 and O26 as most prevalent, the virulence genes were widely distributed among serogroups and impacted by the limitation of methodology. Additionally, it is agreed that new virulent serotypes will continue to evolve, therefore; a need exists for reliable methods to study the relationship between serogroup/serotype, virulence factor variants and host cell specificity.
- In the Canadian perspective, an overview of the November 2010 VTEC Workshop in Quebec was provided. The objective was to develop a basis for rational risk-based food inspection programs for VTEC in the food supply. Specific focus was on the best possible definition of priority VTEC

for testing based on current knowledge and expert opinion and sharing information and expertise on effective, reliable analytical methods for VTEC in foods. From the workshop, the priority VTEC/STEC were narrowed to O157 and 4 to 6 non-O157 serogroups (O26, O103, O111, for most regions, O121 and O145 additionally for other regions and O45 for the U.S.). The best *current* markers for the priority STECs were the *stx* and *eae* genes and serogroup-specific sequences. Other promising targets were also discussed. Finally, several challenges were identified including, but not limited to, the on-going review of the priority VTEC/STEC with public health impact, identification of a marker(s) that determines the presence of priority VTEC/STEC, and effective methods for isolation, differentiation, and identification of priority VTEC/STEC.

Additionally, a panel of six international scientists provided comments based on the presentations that initiated further group discussion.

Panelist included: Peter Feng, Ph.D., FDA-CFSAN; Peter Gerner-Smidt, Ph.D., CDC; Patrick Fach, Ph.D. (AFSSA, France; Mick Bosilevac, Ph.D., USDA-ARS, Ian Jensen, Meat & Livestock Australia; Beatriz Guth, Ph.D., Universidade Federal de Sao Paulo, Brazil.

Key Points:

- It is difficult to make a regulatory classification for pathogenic STECs considering the knowledge gaps in methodology, rapid testing, and the ability of the organisms to acquire virulence factors from mobile genetic elements.
- Increasing use of culture independent diagnostic methods in clinical labs combined with decreasing resources in the public health labs limits the availability of cultures for sub-typing. This may threaten STEC surveillance, outbreak detection, investigation and control, and evaluation of efficiency measures to control STEC in food.
- A need exists to identify common factors for predicting illness as some strains do not have the necessary virulence factors to cause significant illness and thus should not be the focus.
- Testing enrichments for the combination of virulence factors associated with highly pathogenic STEC, will result in fewer samples identified as suspect for STEC.
- A gap may exist in understanding the infective dose for the top STECs and subsequent illness in humans.
- The significance of different non-O157 STEC serogroups remains unclear. The scientific community still needs to know the frequency of these organisms as a group, the specific strains that occur in the animal and in food, as well as their associated virulence factors.

SESSION 2: STEC METHODOLOGY FOR FOODS

Justin Ransom, Ph.D., OSI Industries, co-chair
Nandini Natrajan, Ph.D., Keystone Foods, co-chair

In the second session, technology companies were asked to provide their perspectives on:

- Elements for an effective detection strategy for STECs.
- Diagnostic targets and methods most appropriate for characterization of isolates.
- Advantages and disadvantages to the current detection approach with reference to flexibility in matrix changes.
- Should the target STEC population change, the ability of their respective technologies to adapt.
- Parameters of testing and time to result.

Participating companies included: Qualicon, Pall Gene Systems, BioControl, IEH, and Biomerieux.

Key Points:

- Several companies have developed multiplex real-time polymerase chain reaction (PCR) assays to detect non-O157 STEC in food samples based on target virulence factors or single nucleotide polymorphisms. These methods follow the proposed USDA-FSIS methods.
- Real-time PCR assays are intended to be used as a screen to determine whether or not a sample enrichment contains molecular markers specific to non-O157 STEC that cause human illness.
- Establishments must make a decision to accept or reject fresh product based on the results of a PCR screening test.
- Detection of each marker used to define the presence of a non-O157 STEC strain by multiplex PCR assays does not indicate that all markers are present on the same isolate.
- Culture confirmation on screened colonies would likely be required to confirm that a sample contains a non-O157 STEC isolate for regulatory purposes.
- Some assays to detect non-O157 STEC isolates are commercially available, and others are expected to be validated and marketed in the near future.

Three speakers were asked to provide a global perspective on STEC methods and challenges: Patrick Fach, AFSSA, France; Beatriz Guth, Universidade Federal de Sao Paulo, Brazil; Ian Jenson, Meat & Livestock Australia.

Key Points:

- The potential strategy for food samples could include: *stx*, common intimin (*eae*) types and *nle* for a screening step

and O-antigen associated genes with *eae* subtypes for an identification step.

- The need for selective/differential media exists to narrow the presumptive positives found by some screening methods so isolation confirmation can proceed. Additionally, screening tests must increase the likelihood of identifying enrichments where virulence factors and O-serogroup information resides in the same organism.
- The global need for standard protocols for detection and isolation of non-O157 STEC exists so findings from one investigation or research project can be implemented in other parts of the world. Risk posed by a specific food should be established when it presents a positive PCR result for selected target genes, but isolation was not performed.

Additionally, a panel of six international scientists provided comments based on the presentations that initiated further group discussion.

Panelist included: Pina Fratamico, Ph.D., USDA-ARS; Nick Nickelson, Ph.D., Standard Meat Company; John Ruby, Ph.D., JBS Packing; Roger Johnson, Ph.D., Public Health Agency of Canada; Peter Feng, Ph.D., FDA-CFSAN, and Emilio Esteban, Ph.D., USDA-FSIS.

Key Points:

- A need exists for better traditional testing components: enrichment media, isolation agar, and antibodies for immunomagnetic separation (IMS).
- Three testing platforms may be needed: industry process control test, clinical diagnostic test, and regulatory test.
- The testing platform for non-O157 STECs needs to include O157 so one process can be followed for screening.
- A bank of reference samples may be useful to the industry as research is conducted and methods are validated.

SESSION 3: BREAKOUT SESSIONS

Dane Bernard, Keystone Foods, co-chair

J. O. "Bo" Reagan, Ph.D., National Cattlemen's Beef Association & BIFSCo, co-chair

Nick Nickelson, Ph.D., Standard Meat Company, co-chair

Scott Goltry, American Meat Institute, co-chair
Barry Carpenter, National Meat Association, co-chair

Based on the information shared in the previous sessions, three questions were posed to the attendees. Breakout groups were formed for each question with key points captured and reported back to the larger audience.

Should virulence factor(s) be the basis for pathogenic Shiga toxin-producing *E. coli* detection?

- Yes, at least in part. Virulence genes and other useful unique markers (e.g., single nucleotide polymorphisms) should be the basis in assays to detect pathogenic STEC.
- Virulence genes make challenging targets because they are subject to higher mutation rates and horizontal gene transfer. It is challenging to design primers and probes that are capable of detecting a heterogeneous group of organisms.
- Housekeeping genes are generally more conserved and not affected by horizontal gene transfer but a higher risk of false positives would exist if housekeeping genes were included.
- It is important to keep in mind that a screening test will be able to detect the presence/absence of targets in a broth culture but this does not indicate that all targets are present on a single organism (isolation and screening of colonies would be required to conclusively determine the presence of a pathogenic STEC strain).

List five to seven virulence factors that can be the basis for pathogenic Shiga toxin-producing *E. coli* detection?

- A two-tiered approach is most feasible due to practical limitation of detecting a large number of targets and/or markers on existing platforms.
- Targets suggested to be included in a tier 1 screen included *stx1*, *stx2* (all variants), *eae* (all variants), and O group. *subA* was also suggested.
- Targets suggested for a tier 2 screen included *nleB*, *nleE*, *espK*, *nleG5-2* and *fliC_{H7}*.
- The group discussed the need to characterize pathogenic STEC isolates at the CDC and specifically to characterize those belonging to serogroups other than the top 6.
- The group also described the need to develop assays to subtype isolates into the top 6 non-O157 pathogenic STEC serogroups, *stx2* variants, and *eae* variants.
- The group agreed a need exists for different tests for different intended applications. More specifically, industry needs a rapid test to make a decision whether to accept or reject fresh product within a 12-18 h window. Based on this time restriction, industry must make a decision based on results from a test to screen for the presence of targets in an enrichment. In the public health and academic arena, however, a screening test should be followed up with conventional microbiological culture to isolate and confirm the presence of a pathogenic STEC strain. Also, a clear need

exists to further develop conventional culture methodology (e.g., enrichment media and procedures, immunomagnetic beads, selective and differential agar, and follow-up PCR methods to confirm presumptive positive colonies on plates).

What are the specific research gaps that need to be addressed to support virulence factors as the basis for pathogenic Shiga toxin-producing *E. coli* detection?

- Complete genome sequencing of human clinical pathogenic STEC isolates and comparative genomic studies with sequences representing each seropathotype to identify informative markers for subtyping purposes (e.g., SNPs, variable tandem number repeats, promoter spanning regions, genes or gene fragments).
- Develop a selective and differential media for isolation of pathogenic STEC.
- Develop a database or utilize existing databases (e.g., www.pathogentracker.com) to deposit and share molecular epidemiology data for pathogenic STEC isolates from sectors along the feed/food chain.
- Determine if existing hurdles and interventions during harvest, processing, and subsequent handling that have been established to control *E. coli* O157:H7 are equally effective against non-O157 pathogenic STEC of public health significance.
- Determine the likelihood that a stx-negative strain could be complemented with stx during detection/isolation procedures and during passage through a human host.
- Determine the frequency at which key virulence factors can be gained or lost under conditions designed to simulate host or non-host environments (e.g., experimental evolution).
- Determine how many presumptive positive colonies on an agar plate need to be screened to have confidence that the sample is truly positive or negative for an isolate that carries all the targets that define a non-O157 pathogenic STEC.
- Obtain a better understanding of the putative role of *nle* genes in virulence.

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