Identifying and Controlling *E. Coli* O157:H7 in Processing Plants

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Escherichia coli O157:H7 *(EC)*, first recognized as a potential health hazard in 1982 because of its connection with hemolytic uremic syndrome, bloody diarrhea and neurological disorders, became publicized as a food-borne pathogen in January 1993, following outbreaks in the northwestern United States. Resultant public demand for safer meat products led USDA to propose and to institute changes in the meat inspection system and led fresh meat processors to include warning labels and cooking instructions on meat packages for consumers' use. Although officials believed that this crisis could be controlled rapidly by instituting an integrated HACCP program, developing rapid detection methods, controlling animal hygiene both pre- and post-slaughter, and concentrating on specific products such as ground meats, such actions have not eliminated *EC* as a food-borne pathogen, with subsequent outbreaks occurring across the U.S., presumably from consumption at home of under-cooked ground beef.

This failure by the USDA and meat processors has resulted in proposals by Congress to place all food inspection and regulation under the Health and Human Services (HHS) Department, and by medical associations to conduct more research on the pathogenesis of infections, animal and environmental reservoirs, methods of rapid diagnosis and detection, therapy and prevention, and significance of non-O157:H7 *E. coli* producing the same or similar types of toxin. A National Live Stock and Meat Board panel recommended treating meat carcasses with irradiation, rinsing of meat with effective chemicals, instituting stricter contamination identification methods and educating meat-industry workers and consumers on proper handling methods.

Food-borne pathogen outbreaks can result in high economic annual costs from sickness, death and lost work time. Estimates for the prevalent pathogenic organisms in meat and poultry by cases (thousands), deaths (hundreds), and costs (millions of dollars) are: *Salmonella* (1,920 cases, 9.6 to 19.2 deaths; $600 to $800 costs); *Campylobacter* (2,100 cases; 1.2 to 3.6 deaths; $450 to $500 costs); *Listeria monocytogenes* (1.5 to 1.6 cases; 3.8 to 4.3 deaths; $100 costs); and *EC* (7.6 to 20.5 cases, 1.5 to 3.9 deaths; $100 to $ 300 costs) These estimates include health costs not chargeable to the meat industry but do not include estimates for loss of a firm's reputation and business.

Contamination of carcasses with microorganisms occurs during processing from contact with abdominal contents or feces, transfer from humans or other animals, transfer by aerosols or wash water and contact with unclean processing surfaces. Recent estimates indicate that carcasses can be contaminated up to 26.7% by *Staphylococcus aureus*, up to 15.5% by *Salmonella* and up to 13.3% by *Listeria* or *Yersinia*. Contamination by *EC* from feces of infected animals is believed to be low, but because of the allegedly low numbers of this organism required for infection and the lack of high sensitivity in earlier methods for testing for *EC* in food products, much early data could be flawed toward low incidence values. Since microbial contamination can occur at various times and locations during production, processing, sale and consumption ("pitchfork to dinner fork"), control at various critical points in an integrated production-processing HACCP to reduce incidence of all food-borne pathogens is essential; other possible means for reducing potential or real carcass contaminations include: spray chilling; use of organic acids in alginate gels; washing with trisodium phosphate solutions; and irradiation. Of these, only irradiation (at a dosage of 1.5 to 3.0 kGy and a D^{10} at 0°C) completely eradicates *EC* as well as extending product shelf life by inactivating spoilage organisms. It is, however, unlikely that irradation can be easily or inexpensively adopted for treatment of all meat products. Effectiveness of irradiation is dependent upon processing temperatures and the stage of microbial growth; log phase cells are more sensitive than stationary phase cells and frozen meat is more resistant than unfrozen.

The most promising method presently appears to be HACCP, with identification of critical points for possible pathogen contamination (including identification of infected animals, carcasses and meat products) and alteration of procedures at those points to control microbial entry and growth, with quality of raw materials being especially critical. Essential for HACCP is the development of accurate methods for identifying *EC* when present in the animal, carcass or meat product. Although reported as being isolated from ham, turkey, raw milk, venison and water, *EC* appears to be most prevalent in ground beef products. Identification of infected animals has been difficult in that this organism, although believed to be always present, may be isolated from those animals only at certain times; which may result from a shedding of *EC* only under stressful conditions, possibly during transport to slaughter. The organism binds strongly to Type I collagen present in connec-

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Reciprocal Meat Conference Proceedings, Volume 47, 1994.

tive tissues, skin and hide, muscle endomysial and epimysial sheaths and adipose tissue membranes, giving the organism many sites on the meat surface for attachment, and allowing transfer from such surfaces during processing.

Methods for Identifying *EC*

Modern molecular biochemical techniques now permit development of methods for isolation and identification of selected microorganisms from various substrates, including food products. When used in the food industries, such improved methods increase the real and implied food quality and safety through early detection and elimination of pathogenic organisms and prevention of food-borne disease outbreaks. Many of these microbial isolation and detection methods were designed primarily for use in medical fields to furnish more accurate data for treatment of diseases and to monitor outbreaks, sources and mode of transmission. Consequently, some methods must be adapted from medical to food-industry environments. Both fields require tests that are accurate, specific and sensitive to low levels of organisms. Also, development of rapid simple tests that could be automated is quite desirable. Tests conducted in-house for the food industry, however, should also be non-hazardous to avoid contamination of the product or employees, and cost-effective for use in small- or medium-sized processing plants.

Most earlier methods for detection and identification of microorganisms isolated from food or other sources depended upon an organism being viable. Each isolate was given a history which included the sample source, media requirements, its growth on selective media, atmosphere and temperature needs and colony characteristics. The organism was identified under a microscope for shape and size, motility and presence of flagella, presence of spores, surface characteristics and Gram-staining properties. Biochemical classifications included carbon utilization, metabolic characteristics, identity of exported enzymes, toxins and other products, fatty acid profiles, DNA base composition, immunological classifications and phage typing.

Recent microbial nucleic acid tests that do not require viable cells for most analyses include the isolation and identity of nucleic acids from the plasmids, phages, chromosomal DNA and ribosomal-associated RNA's. The parent *E. coli* organism is an aerobic and facultative anaerobic, motile or non-motile, catalase (+), non-sporing, Gram-negative, short straight rod usually found in intestinal sites of warm-blooded animals. Optimum temperature is near 37°C, but it has a fairly wide range for growth. Although most strains of *E. coli* are non-pathogenic, the pathogenic strains have been classified into 4 classes: entero-toxigenic *E. coli* (ETEC), entero-invasive (EIEC), entero-pathogenic (EPEC), and entero-hemorraghic (EHEC). *EC* has been assigned to EHEC, a former sub-group of EPEC. This organism is classified by its antibody reactions with somatic antigens (O represents the German *ohne hauch* [without movement]) and flagellar antigens (H represents the German *hauch* [with movement]). Earlier methods for isolation failed to pick up this strain; it has poor growth at 44° to 45.5°C, a common temperature for isolating intestinal coliforms (but growth is improved in higher ionic strength media); it does not ferment sorbitol in 24 hrs; and it is negative for beta-galactosidase, an enzyme detected using a fluorescent derivative in the common MUG assay for *E. coli*. Recently, virulent sorbitol positive strains have been isolated, which may hinder isolation of such *EC* when a sorbitol medium is used. Cellular virulence factors include: presence of a 60 MDa plasmid; and the production of a shiga-like toxin or verotoxin, an enterohemolysin, and an exported protein *intimin* regulated by a 97 KDa gene *eae* (for *E. coli* attaching and effacing). The present *EC* appears not to have been descended from other O157 strains but clonally from an 055 strain and to have developed its virulence from incorporation of the various factors by transfer from other organisms. Although *EC* does not show an increased resistance to heat lethality, being more heat-sensitive than *Salmonellae* (but heat shocking raises the temperature resistance), it seems fairly resistant to lowered pH or acid washes.

Specific assay methods for organisms require a separation of desired cells from other cells present, often an enrichment to increase numbers, followed by qualitative or quantitative determinations. Each procedure requires a finite period which can extend time required for detection and positive identification. Various preparation procedures include homogenization, blending, or stomaching the meat for releasing attached cells, and centrifugation or filtration to physically separate the microbial cells from the mixture. For isolating presumptive *EC* from this mixture, methods have used: Initial dilutions on selective media (such as Mac Conkey Agar with added sorbitol); physical separation using magnetic immunobeads; or selective membranes, e.g., hydrophobic grid or petrifilms. Presumptive isolates are usually enriched by growth in a rich nutrient recovery medium for 12 to 24 hours to enhance the sensitivity of the assay, followed by the specific test.

Polymerase chain reaction (PCR) techniques can be used with non-viable cells for amplification of selected microbial nucleic acid fragments, but this method is not easily adaptable to routine food product analyses. It might be used when determining critical control points to detect non-culturable organisms present prior to processing.

Present *EC* tests in the food industry have been designed for optimizing convenience, speed, reliability, computerization, miniaturization, portability, automation and safety. All tests, however, should always be validated with certified microbial isolates as positive controls. Four tests were demonstrated during the session: An ELISA method (EHEC-Tek™ test kit); a hydrophobic membrane filter for enumeration (ISO-GRID™); an immunoblot assay method for colonies isolated on petrifilm plates (Petrifilm Test Kit HEC); and a coline visual line immunoassay using a dipstick (AMPCOR). Known isolates were used for demonstrations, so normal times for isolation on selective and/or enrichment media which may take an additional 18 to 24 h were not included. Other assays presently available or being developed use ELISA or latex agglutination steps on the selected isolates.

Control of *EC* and related toxic bacteria in meat products will require an integrated HACCP program that regulates quality of raw ingredients and the contamination of product during processing. Identification of infected animals may be difficult because of the noted variabiliity in shedding rates. Since *EC* is inactivated by normal temperature regimens for processed meats, HACCP efforts should be directed toward fresh meat

products, particularly ground meats, to eliminate contamination of meat and equipment surfaces. As present tests may require 1 to 2 days for a presumptive detection and up to 6 days for positive confirmations, many fresh products may have been marketed and consumed. Any suspicion of a contamination by *EC* should require immediate steps to determine the source of contamination and means for elimination to prevent further outbreaks. Valid assays and proper record-keeping are essential.

NOTE: An expanded version of the manuscript with references is available from Dr. Benedict.