AMERICAN MEAT INSTITUTE
Serving The Industry Since 1906.

(76)

RECEIVED
FSIS DOCKET ROOM
99 APR -6 PM 3:45

April 5, 1999

Thomas Billy
Administrator
Food Safety and Inspection Service
Room 331-E Jamie L. Whitten Building
14th & Independence Avenue
Washington DC, 20250

97-068N
97-068N-76
Kimberly K. Rice

Dear Mr. Billy:

During the March 8, 1999 Public Meeting on Beef Products Contaminated with *Escherichia coli* O157:H7 (*E. coli* O157:H7 or the pathogen), the industry coalition agreed to submit a pilot test protocol that would provide FSIS with information to support the coalition's recommendations for changes to Directive 10,010.1, *Microbiological Testing Program for Escherichia coli O157:H7 in Raw Ground Beef.* A copy of that protocol is attached hereto.

This pilot test must be considered in the context of broader research approach industry is planning regarding *E. coli* O157:H7. Several projects have been developed to help the industry find a solution to the *E. coli* O157:H7 problem. Those projects include developing rapid methods for detection of the pathogen in raw beef products, developing optimum carcass and hide sampling methods to test for *E. coli* O157:H7, determining the incidence and ecology of *E. coli* O157:H7 on cattle entering slaughter facilities to determine the effect on carcass contamination, and developing treatment methods to reduce the pathogen's incidence in live animals.

The attached protocol is only one piece of this global research approach and it does not answer all the questions involving the pathogen. The protocol, however, will enable FSIS and the industry to establish a system to verify routinely slaughter plant interventions used to control *E. coli* O157:H7 contamination on carcasses and to define a

Post Office Box 3556, Washington, DC 20007 · 1700 North Moore Street, Arlington, VA 22209
Phone: 703/841-2400 Fax: 703/527-0938 Http://www.meatami.org

reliable means for reducing the risk of *E. coli* O157:H7 in beef. We look forward to discussing the protocol and next steps in this process with you and your staff.

Sincerely,

Kimberly K. Rice

Kimberly K. Rice

Attachment

PILOT TEST PROTOCOL FOR E. COLI O157:H7 ON BEEF CARCASSES

Introduction

In January 1993, a severe outbreak of foodborne illness occurred in the Pacific Northwest of the United States. This particular outbreak was associated with the presence of the pathogen *E. coli* O157:H7 in undercooked ground beef. As a result of this incident, the Food Safety and Inspection Service (FSIS) on October 17, 1994, implemented a microbiological testing program for *E. coli* O157:H7 in ground beef. FSIS Directive 10,010.1 *Microbiological Testing Program for Escherichia coli O157:H7 in Raw Ground Beef* states, "The objective of the testing program is to test for *E. coli* O157:H7, and to stimulate industry action(s) to reduce the presence of the pathogen in raw ground beef." Since the implementation of Hazard Analysis Critical Control Points (HACCP) system, slaughtering and processing plants subject to the regulation are required, in their HACCP plan, to identify processing measures being used to prevent fecal and ingesta contamination on carcasses and to control the presence of *E. coli* O157:H7. Recently, FSIS in their policy clarification (64 FR 2803, Beef Products Contaminated with *Escherichia coli* O157:H7, January 19, 1999) expanded the definition of adulteration to non-intact beef products including injected beef and trimmings for *E. coli* O157:H7 testing. This expansion was the impetus to redirect industry efforts.

The purpose of this testing protocol is to establish a system that would routinely verify slaughter plant interventions for controlling *E. coli* O157:H7 contamination on carcasses, define a reliable means for reducing the risk of *E. coli* O157:H7 in beef and allow USDA to reduce sampling of all beef products at federally inspected establishments and retail stores.

Survey Design

A study will be conducted in at least twelve slaughtering plants nationwide (approximately six plants that slaughter fed steers/heifers and approximately six plants that slaughter non-fed cows/bulls). Sampling for *E. coli* O157:H7 will occur at a rate of one per 300 carcasses for *E. coli* O157:H7 at three points in the slaughter process: 1) before hide

removal; 2) prior to carcass wash; and 3) after application of microbial intervention(s). Each selected animal will be tagged with an identification code and tracked through the slaughter process. After the animal is stunned and bled, the hide will be sampled. After hide removal, one side of the split carcass will be randomly selected and sampled prior to carcass wash. The remaining side of the carcass will be sampled after application of microbial intervention(s). Alternate sides of the carcass will be sampled prior to carcass wash and after application of microbial intervention(s). Slaughter plants participating in the survey must use at least one whole carcass microbial intervention system such as organic acid rinses or thermal processes. Hide and carcasses will be sampled for *E. coli* O157:H7 to verify the effectiveness of intervention systems for a period of four weeks. Purchasers of trimmings from participating plants will continue to sample trimmings according to their established protocols during the four week pilot test.

Hide Sampling Procedures

Moisten sterile sponge by adding 10 ml of Butterfield's phosphate diluent (BPD) onto a sponge and then sponge a 4 inch X 10 inch area on the brisket (anterior to the navel or ventral midline) at the site where the hide opening pattern marks are made. Place sponge in a sample Whirl-Pak bag and add 15 ml of BPD to bring the total volume to 25 ml. Remove excess air from the bag before closing. Following collection, refrigerate all samples and place in cooler with ice packs for shipment by overnight air express to a designated laboratory for analysis.

Carcass Sampling Procedures

A composite sponge sample will be taken at three different sites on the carcass side as prescribed in USDA's Pathogen Reduction/HACCP rule. The sites will be immediately adjacent to the hide opening pattern marks and include: 1) brisket - anterior to the navel or the ventral midline; 2) flank - posterior to the navel or the ventral midline; and 3) rump – the cushion of the round.

Moisten sterile sponge by adding 10 ml of Butterfield's phosphate diluent (BPD) onto the sponge and then sponge a 4-inch X 10-inch area on carcass flank. The pressure of the sponging should be constant (approximately 5 lbs pressure) with each sample. Move to brisket area and proceed sponging using the same side of the sponge for the flank. Move to the rump and with the other side of the sponge. Place sponge in a sample Whirl-Pak bag and add 15 ml of BPD to bring the total volume to 25 ml. Remove excess air from the bag before closing. Following collection, refrigerate all samples and place in cooler with ice packs for shipment by overnight air express to a designated laboratory for analysis.

Testing and Holding Carcasses

All sampled carcasses will be separated and visibly marked and held until test results are confirmed negative. Any carcass sampled after application of microbial interventions that is confirmed positive for *E. coli* O157:H7 will be rendered or used in cooked items that are subjected to a thermal process that destroys *E. coli* O157:H7. If a post-intervention carcass tests positive for *E. coli* O157:H7 the plant will investigate the cause by reviewing their slaughter procedures and carcass intervention systems.

Microbiological Analysis of Hide Samples

A sponge sample will be placed in an individual plastic bag with 90 ml of GN+A (Gram Negative Broth + Antibiotic) and stomached for 2 minutes. Transfer 1 ml aliquot to microcentrifuge tubes containing immunomagnetic beads coated with *E. coli* O157:H7 antibody (Dynabeads, Dynal, Inc.). Incubate at room temperature for 30 minutes with continuous mixing. The use of a rotating device is recommended. Insert the magnetic plate into the Dynal MPC-M®. Invert to concentrate the beads onto the side of the tube. Allow 3 minutes for proper recovery. Carefully remove the supernatant as well as the remaining liquid in the tube's cap. Remove the magnetic plate from the Dynal MPC-M®. Add 1 ml of washing buffer (PBS-Tween), and invert the Dynal MPC-M® three times to resuspend the beads. Resuspend the bead-bacteria complexes

in 100 µl of PBS-Tween and whirl mix. After immunomagnetic separation, transfer 50 µl of the resuspended beads onto a CT-SMAC. Spread the bead-bacteria complexes on half of the plate by using a standard microbiological sponge, and dilute further be streaking with a loop. Incubate the plates for 24 hours at 37°C. Pick presumptive colonies of *E. coli* O157:H7 (can test colony immediately with Meridian Test) and isolate for further confirmation.

Microbiological Analysis of Carcass Samples

A sponge sample will be placed in an individual plastic bag with 100 ml of Modified-EC broth with novobiocin and homogenized by a stomacher for 30 sec and incubated for 18 to 24 h at 35°C. Transfer 0.1 ml enriched broth into the sample addition well of the VIP device and start the initiation of a lateral flow of broth along the surface of a solid support. During initial hydration of the device, EHEC reacts with an antibody—chromogen complex contained in the device. If EHEC is present, a detection line forms that is positioned across the solid support in a viewing window of the device, indicating a positive reaction. A procedural control window also exists wherein a second line is formed, indicating proper test completion. Absence of a procedural control line indicates an invalid test. After enrichment, total assay time is about 10 min from the time incubated broth is added to the test well.

Confirmation Test for *E. coli* O157:H7

Before plating the Modified-EC broth on BCM, concentrate the *E. coli* O157:H7 by treating the broth with immuno-capture beads. Follow package directions for using the beads. Serially dilute concentrated beads 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. Plate 0.1 ml of 10^{-2},10^{-3}, 10^{-4} dilutions onto BCM agar plates. Use bent glass rod to evenly distribute inoculum over agar surfaces. Incubate overnight at 35°C. Examine the plates for typical/atypical colonies. Typical colonies are dark blue to black, domed to raised colonies 1.5 – 2.5 mm diameter, with a black precipitate surrounding/under the colony. No clear ring around the colony. Atypical colonies are blue to turquoise in color with a blue/black precipitate surrounding/under the colony. Pick 12 well isolated suspect colonies. Streak each of the 12 colonies to the

Reference

Food Safety and Inspection Service. 1996. Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems. U.S. Department of Agriculture, Washington, DC.