Management of Pathogens in Fresh Meats Processing

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During November 1992 to February 1993, over 500 people in Washington, Idaho, California and Nevada suffered infections caused by *Escherichia coli* O157:H7. Four deaths were associated with this outbreak. This foodborne disease outbreak was linked to the consumption of improperly cooked hamburger meat. This unfortunate incident has heightened the need for information by consumers, producers, meat processors, regulators, food service operators and food scientists. This presentation is designed to provide a general overview of the most important features of *E. coli* O157:H7 and means of its control.

The first documented outbreaks of hemorrhagic colitis caused by *E. coli* O157:H7 occurred in Michigan and Oregon in 1982. A total of 47 cases were attributed to undercooked hamburger meat. Since that time, additional outbreaks have been reported implicating raw milk, ground meat, person-to-person transmission, ham-turkey-cheese sandwiches, potato, turkey roll and apple cider. Outbreaks from *E. coli* O157:H7 have been reported worldwide.

Escherichia coli are common bacteria found in the intestinal tract of man and animals. This diverse group of bacteria includes thousands of strains, most of which are harmless to humans and the animals they inhabit. These organisms are commonly tested for in foods and water as indicators of fecal contamination. Unlike non-pathogenic *E. coli*, O157:H7 is MUG negative, will not grow at 44.5° to 45.5°C, and is sorbitol-negative. *Escherichia coli* O157:H7 is an enteropathogenic *E. coli* (EEC) and more specifically referred to as enterohemorrhagic (EHEC). Other enteropathogenic *E. coli* include: EPEC (enteropathogenic), usually a disease producer in neonatals with adults as carriers; EIEC (entero-invasive), which is similar to *Shigella*, does not produce gas in glucose, and is the 0124 group; and ETEC (enterotoxigenic), which causes traveler's diarrhea and is similar to cholerae. There are several toxins produced by the various enteropathogenic *E. coli* groups. For simplicity sake, the O157:H7s produce verocytotoxins and are sometimes also designated as VTEC.

The disease caused by *E. coli* O157:H7 is characterized by frank or pure blood in stools, very severe abdominal pain, some vomiting, but no fever. Time of onset is three to four days. Duration is two to nine days with an average of four days.

Hemorrhagic colitis is the first stage of the disease. In children, this can turn into the rare but fatal disorder of "hemolytic uremic syndrome" (HUS). In adults, it may progress to "thrombotic thrombocytopenic purpura."

Escherichia coli O157:H7 is associated with the intestinal tract of warm-blooded animals. Cattle are considered one of the main reservoirs, since *E. coli* O157:H7 has been isolated from feces and raw milk. The organism has also been isolated from retail samples of beef, pork, lamb, chicken and venison. The incidents involving potatoes and apple cider have potential animal fecal contamination history.

There is a need for more rapid and specific methodology for isolation and identification of *E. coli* O157:H7 from food materials. Currently, a culture procedure recognized by the United States Department of Agriculture (USDA) involves 24 hours of pre-enrichment incubation followed by plating onto a differential medium which is incubated for 24 h. Thus, the minimum time for a negative result is 48 h. If suspect colonies are present on the plating medium, a minimum of 12 colonies must be picked and examined, requiring an additional three days. A more rapid method utilizing an "immuno-blot" membrane filter procedure is also recognized by the USDA. This procedure can produce negative results in 26-28 h. Confirmation of presumptive positives requires an additional three to four days. Experience with both the immuno-blot and USDA culture method suggests that many samples are presumptively positive and require confirmation. An Enzyme-Linked-Immunoassay (EIA) procedure utilizing a monoclonal antibody has been developed and will soon be commercially available, and research is underway to develop genetic probe methods. These new methods may reduce the time of analysis and provide improved specificity and sensitivity.

Escherichia coli O157:H7 is no more heat-resistant than *Salmonella* and, from data reported in the literature, is probably less heat-resistant. Table 1 lists time/temperature combinations which have been recommended by the National Advisory Committee on the Microbiological Specifications for Foods (NACMSF) for inactivation of *E. coli* O157:H7 in ground beef. Since these time/temperature recommendations require expensive monitoring equipment, visual guidelines such as "cook until gray or brown in the center until the juices from the meat are clear" are being suggested. *Escherichia coli* O157:H7 has been shown to survive frozen storage for up to nine months at –20°C.

NACMSF also made other recommendations to prevent *E. coli* O157:H7 ground beef-associated infections in humans. The following is a summary of their recommendations to the USDA and Food and Drug Administration (FDA).

NACMSF Recommendations

1. Expedite immediate initiation of Hazard Analysis and Critical Control Point (HACCP) programs by slaughter opera-

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tions, processors, food service and retail industries and consumer education.
2. Label all raw and partially cooked products to indicate proper refrigeration, handling and cooking.
3. Include intervention strategies such as carcass rinse to HACCP programs for slaughter and processing.
4. Endorsement of a 5D cooking requirement (Table 1).
5. The development of practical guidelines on cooking requirements by the Food Safety and Inspection Service and FDA.
6. More research on the ecology of *E. coli* O157:H7 and rapid means for identifying animals and beef products which harbor the microorganism.

Management of *E. coli* O157:H7 in fresh meats will be accomplished through HACCP systems from the farm to consumers. However, much remains to be learned in order to adequately identify hazards and appropriate control procedures. Currently, it is wise to assume all fresh meats may potentially contain the organism and should therefore be properly cooked. Further, cross-contamination from raw to cooked product must be prevented.

Table 1. NACMSF Time/Temperature Recommendations for Inactivation of *E. coli* O157:H7 in Ground Beef.

Temperature		*Time*
°C	*°F*	*(minutes)*
60.0	140	8.34
62.2	145	2.11
65.6	150	0.53
68.3	155	0.13

Discussion

R. Frechette: Have we identified any asymptomatic human carriers yet?

M. Doyle: Yes sir, there was a study done in Minnesota in a day-care center where they have identified asymptomatic human carriers. There are different degrees of illness and in some cases it's just watery diarrhea, you don't have to have full-blown bloody diarrhea.

Frechette: Have we found any workers in the slaughter facilities coming down with O157:H7 infections? One study told us about where people were picking it up off of the potatoes. Why haven't we seen it in the slaughter facilities?

Doyle: I am not aware of any cases where an individual has picked up the organism handling cattle during slaughter; but because we haven't found it or made that association, we cannot rule out the possibility.

Frechette: One last question, in Germany they're reporting O157:H7 infections which are sorbitol positive. That is one of the first screens we use to identify or to isolate it. Has this been confirmed in this country, or is it specific to Germany?

Doyle: I haven't received those isolates. In talking to a colleague who has received one, apparently they are not readily accessible; it's not an O157:H7, it is a 0157 non-modal. It can cause hemorrhagic colitis and hemolytic uremic syndrome as well. That is the kind of isolate that they are finding in Australia and South Africa also. It is not the same as the O157:H7 which typically is sorbitol negative.

W. Henning: Some of the surveys shown where they had identified 0157 in some of the calves, the CDC reported that six weeks later, those same calves tested negative. Do we know much about the condition it takes to grow this organism? Is there a certain pH, or anything else that would help us control it?

Doyle: That's part of the reason for this follow-up survey being done by USDA-APHIS. It is to provide questionnaires to farmers so that there may be an opportunity to correlate certain management practices with those animals that carry the organism. Secondly, we would also like to do a follow-up study to monitor those calves that are positive to see how long they shed. We would like to necropsy those animals after they are no longer excreting the organism to see if the organism is still present and, if under stressful conditions, can be stimulated to shed the organism again. These types of studies are underway. We are not the only one doing these types of studies. I know Bob Clark at Ag Canada is doing some of this work, as well as Dale Hancock and Dr. Besser at Washington State.

J. Carpenter: What is the cost per sample for analyzing this particular *E. coli?*

Nickelson: I guess the quotes are probably $20 to $35 per analysis at most commercial labs. Additional cost to serotype O157:H7 is double that in most cases.

Carpenter: Bo, I wanted to ask you if they considered the use of propionic acid in the organic acid rinse. In some work that I did supported by the American Meat Institute Foundation about 25 years ago, we used propionic acid in combination with some of the other organic acids and it was more effective than using just acetic or citric acid.

J. Reagan: We looked at that, one of the things that we considered was concentration level. We knew it had to be very weak (3/4 to 1-1/2%), I think your work used concentrations a little bit higher than that. We realize we need to do additional studies which look at combinations of acids. There are combinations being used today, mostly lactic and propionic, which are about half the cost, I think. There is evidence in the literature that lactic acid works better than anything else at 1 to 1-1/2% but cost becomes a factor. Some people are currently using combinations of lactic and propionic to reduce costs.

D. Allen: I had a statistician look at the probability of contaminated product with O157:H7 using 0.05% to 0.5% infected herd level with a 10% to 100% effectivity level in preventing the contamination. With a worst-case scenario, if we are 90% effective in not contaminating and the organism exists at a 0.5% level in the herd, as some studies suggest, we still have

4% of our product contaminated. If you are at that level and are totally ineffective, every time you have it you contaminate, we have 1/3 of our product contaminated. With that number of incidences, and when I hear from various sources that they've tested 2 to 10 thousand times and not found it, there is a hole in the data. Would you care to speculate where the hole is?

Doyle: We have found that testing for this organism takes a lot of time. Not all laboratory procedures are equivalent. I am hearing the same thing where they've tested 2 to 3,000 samples and they are all negative. But it's funny, we went to Georgia and looked at milk after it was suggested that the organism was not in the south, but it's there. We find it there when we look for it. I think its a matter of how hard you look, a matter of methodology, and personnel actually doing the testing.

Nickelson: I think we do a better job at preventing contamination in the plants than we take credit for. The other thing is methodology. In some of the initial surveys, samples were direct plated on MacConkey Sorbitol with no enrichment. Competitive floras were probably preventing identification of O157:H7. Picking colonies is also a factor. We know that with Listeria monocytogenes, if you pick 5 colonies you may not find a positive. If you pick 10, you may not find a positive; but, if you pick 20 colonies, the incidence rate will increase almost 30%. Therefore, I think it's a matter of who is looking and the sophistication of the analysis being used. There are a wide variety of tests being used. I know USDA is planning to solicit soon in the Federal Register a rapid procedure to be used in future USDA testing.

Doyle: I might add that when we tested the fecal samples from these calves, the 19 to 20 calves that were positive, only half were positive by direct plating, all were positive by enrichment. So if we had only done direct plating, we would have missed 50% of the positives.

P. Husband: On the subject of the organic acid sprays, I have been told that they are only allowed to be used for pre-evisceration situations, and not for post-evisceration. Is that true? Is there any reason? And has any thought been given here to the use of the hot water decontamination to achieve the same ends?

Reagan: Organic acid sprays are allowed both pre- and post-evisceration. Hot water decontamination is allowed at temperatures not to exceed 55°C.

Allen: What do we do with this product when we find contamination?

Nickelson: I agree with you that we have probably overreacted to this thing. I think we might want to monitor for O157:H7 just like we monitor for salmonella, to get an idea of the overall cleanliness of an operation. But just like salmonella, we can't start dumping meat because the meat is salmonella positive. If you look for it, you're going to find it. It's going to take a long time to convince some suppliers not to put negative salmonella into their specs but to put some kind of incidence level, such as, less than 5% of samples examined being positive. I think that's what we will have to do with O157:H7 as well. It's going to take a tremendous education program to do that.

D. Kropf: I'm going to raise another issue that concerns us a great deal. This deals with cooking appearance. Some of you know that last year at the food safety consortium, we reported this and it is now submitted for publication. We have found that there some sources of ground beef that when cooked to temperatures monitored by a hypodermic needle probe, can be cooked to very low temperatures and appear well done. We have cooked some ground beef patties to 55°C, (131°F), that looked to be very well done samples. The other issue that was brought up, USDA says if juices run clear, meat is safe; we would challenge that also. I think we would rather say that if juices lose their pink color, which we found they do about at 77°C, then that is an assurance of temperature. I think that we are going to have to work with people who prepare ground beef to assure the cooking time and temperatures are enough to assure us safety. I would welcome any comments.

Nickelson: I think most people who were trying to react to the situation, realized that the recommendation to cook until juices run clear was a short-term fix. Most organizations who have tried to make sound recommendations based on scientific results have said we do not have good information on cook times and temperatures and temperature monitoring and that research dollars need to be applied to these problems.

Reagan: I agree. When we look at proposals this year, temperature monitoring will be key. I think we need to come up with a better way of monitoring and a better indicator of when the end point temperature is reached. Again, I think we are in a different place today than we were last November. The significance of your preliminary work is much more meaningful today than 12 months ago. I would encourage you guys to keep working on that and continue to get the work out.