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ABSTRACT

Significant effort has been targeted at reducing the risk of pathogens in U.S. beef products since the mid-1990s. These efforts were focused on *Escherichia coli* O157:H7 after it was declared an adulterant in ground beef or its components. Post-harvest interventions applied to hides and carcasses by beef processors resulted in significant progress. Effective pre-harvest approaches proved hard to identify and implement. Six additional pathogenic *E. coli* serogroups were made adulterants in some beef products in 2012 and discussion regarding *Salmonella* is ongoing. Success to date has resulted from the combination of regulatory, research, and industry efforts to reduce the presence of pathogens in beef.

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1. Introduction

The 1992–1993 outbreak of *Escherichia coli* O157:H7 in the Western United States (Centers for Disease Control and Prevention [CDC], 1993) began an era of intense effort to reduce the risk of this pathogen in the red meat supply. The U.S. meat industry and government have invested millions of dollars in research to control *E. coli* O157:H7. These efforts have been very successful, as demonstrated by the United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) data on *E. coli* O157:H7 testing showing a decline to <0.25% positive samples (Table 1) and CDC reports of *E. coli* O157:H7-related illnesses, which have declined from 2 per 100,000 population in 2000 to 1 per 100,000 population in 2011 (Table 2). *Salmonella*-related illnesses have changed very little over time ranging from 13.5 to 17.5 per 100,000 population (Table 2). The Healthy People 2020 goal is 0.6 *E. coli* O157:H7-related illnesses per 100,000 population and 11.4 *Salmonella*-related illnesses per 100,000 population (DHHS, 2014). Although CDC data represents total illnesses, not just those attributable to beef consumption, they are consistent with FSIS trends of reduced positive tests for *E. coli* O157:H7. This progress by the beef industry is the result of a combination of increased regulatory requirements by FSIS, implementation of research results on interventions and novel findings about how and where pathogen contamination occurs, an increased focus on best practices by the industry, and perhaps most importantly, the sharing of information that facilitated all of the above.

There are a number of key events/milestones that have played a pivotal role in the increased control of *E. coli* O157:H7 in the U.S. meat supply. These were first described by Koohmaraie et al. (2007) and the list has grown since then. Here we will separate them into key industry events/decisions and novel research findings. Each of these has contributed greatly to the collective success of the beef industry's control of pathogens in the U.S. red meat supply. The 1992–1993 multi-state outbreak of *E. coli* O157:H7 focused everyone's attention on *E. coli* O157:H7 and brought about enforcement of zero tolerance for *E. coli* O157:H7 by FSIS and a subsequent declaration of it as an adulterant in ground beef and trim. This was followed closely by mandated Hazard Analysis and Critical Control Point (HACCP) regulation (CFR, 1996), and later requirements for in-plant validation of interventions. Recently, six non-O157 Shiga toxin-producing *E. coli* (STEC) serogroups were added to the list of adulterants in beef (O26, O103, O111, O145, O45, O121) which brought renewed attention to the efficacy of antimicrobial interventions. The Beef Industry Food Safety Council (BIFSCO, 2014) was formed to allow industry leaders, beef companies, and food safety researchers to come together in various forums to find solutions to problems. The resulting knowledge sharing within the industry, in the form of various conferences, task forces, councils and summits, all greatly facilitated problem solving. Perhaps, the most significant role of BIFSCO has been adoption of industry-wide decision to agree that food safety was a non-competitive area and to encourage collaboration and

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Table 1
USDA Food Safety and Inspection Service Microbiological results for raw ground beef and raw ground beef components combined analysis for *E. coli* O157:H7 or non-O157 STEC.[a]

Year	Number positive	Number tested	% positive
1994	0	891	0.00
1995	3	5407	0.06
1996	4	5703	0.07
1997[b]	4	6065	0.07
1998	14	8080	0.17
1999[c]	32	7785	0.41
2000	35	5819	0.60
2001	49	6356	0.77
2002	40	6241	0.64
2003	18	6409	0.28
2004	14	7959	0.18
2005[d]	19	10,963	0.17
2006	20	11,755	0.17
2007	27	12,225	0.22
2008[e]	49	11,183	0.44
2009	40	12,293	0.33
2010	27	12,225	0.22
2011	23	16,352	0.14
2012	45	16,262	0.24

[a] Includes only initial random sampling, no follow-up samples.
[b] During October 1997, the amount analyzed was increased from a 25 g sample to a 325 g sample to provide increased detection sensitivity.
[c] On September 3, 1999, a new selection and detection method was introduced to further increase test sensitivity.
[d] During October 2005, a new screening method was introduced to reduce the number of screen positives that do not confirm positive.
[e] Beginning with 2008, annual microbiological sample results will be posted according to the date the sample was collected. Prior to 2008, yearly posting of microbiological data results was based upon the sample analysis completion date. For this reason, data from 2008 cannot be directly compared to 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.

information sharing between competing companies. BIFSCO has taken the lead on industry educational efforts and periodically updates best practices' documents (BIFSCO, 2014).

Numerous antimicrobial interventions were demonstrated efficacious and implemented beginning in 1994 and investigations to improve interventions have continued to date (Koohmaraie et al., 2005, 2007). One of the more significant findings was the demonstration that the hide was the primary source of carcass contamination (Barkocy-Gallagher et al., 2003; Elder et al., 2000; Nou et al., 2003; Small, Wells-Burr, & Buncic, 2005) and the subsequent development of hide-on carcass washing systems feasible for large and small operations alike (Arthur, Bosilevac, Brichta-Harhay, Kalchayanand, King, et al., 2008; Arthur, Bosilevac, Brichta-Harhay, Kalchayanand, Shackelford, et al., 2007; Bosilevac et al., 2004; Bosilevac, Shackleford, Brichta, & Koohmaraie, 2005). Other significant research outcomes include development of rapid tests for detection of pathogens that facilitated test-and-hold programs, whereby beef trim was not released into commerce until the test for *E. coli* O157:H7 was found to be negative. Research to reduce the cost of test-and-hold and consensus that N60 was the best available trim sampling approach led to wide-spread voluntary adoption of test-and-hold programs which FSIS has proposed to make mandatory. The N60 sampling plan is based on a statistical calculation that if the average incidence of *E. coli* O157:H7 is 5%, then 60 random subsamples from the lot pooled and tested gives 95% confidence of detecting a positive lot (Anonymous, 1986).

Table 2
CDC[a] data on *E. coli* O157:H7 and *Salmonella* related illnesses per 100,000 population.

Year	O157	*Salmonella*
1997	2.1	13.6
1998	2.4	13.6
1999	1.9	16.1
2000	2.0	14.1
2001	1.6	15.0
2002	1.7	16.2
2003	1.1	14.4
2004	0.9	14.6
2005	1.1	14.5
2006	1.2	15.0
2007	1.2	15.0
2008	1.2	15.0
2009	1.0	15.0
2010	0.9	17.5
2011	1.0	16.4
2020 goal[b]	0.6	11.4

[a] Center for Disease Control and Prevention.
[b] Healthy People 2020 goals (DHHS, 2014).

More recent results have brought development of pathogen enumeration protocols that were logistically and economically feasible to be used on large numbers of samples (Brichta-Harhay et al., 2008) that enabled the critically important routine determination of levels of pathogens. Enumeration of the pathogens revealed the importance of even simple hide-wash interventions (Arthur, Bosilevac, Brichta-Harhay, Kalchayanand, et al., 2007; Arthur, Bosilevac, et al., 2008) and the contribution of "supershedder" cattle to contamination of all hides (Arthur, Brichta-Harhay, Bosilevac, Kalchayanand, Shackelford, et al., 2010; Arthur et al., 2009; Cobbold et al., 2007; Matthews et al., 2006; Omisakin, MacRae, Ogden, & Strachan, 2003). Animals that are shedding a high level of a pathogen in their feces (at least 10^4 CFU/g) have been called supershedders and may be the source of a majority of pathogen contamination in their pen. The addition of six more STEC declared adulterants in beef and increased discussion of regulating *Salmonella* in some manner, resulted in additional research to evaluate antimicrobial interventions used to reduce them (Arthur, Kalchayanand, Bosilevac, Brichta-Harhay, Shackelford, et al., 2008; Geornaras et al., 2012; Kalchayanand et al., 2012).

As control of *E. coli* O157:H7 increased resulting in fewer sporadic positive *E. coli* O157:H7 tests, a phenomenon known as High Event Periods (HEP) has become the focus of pathogen reduction efforts (Arthur, Bono, & Kalchayanand, 2014). These HEP are when an unusually high number of trim samples test positive for *E. coli* O157:H7 in a short time period, usually within one shift or day of production that start usually with no discernible breakdown in the food safety system and end with no corrective action implemented. Another significant research finding was that lymph nodes could harbor *Salmonella* and may be a significant source of *Salmonella* that end up in ground beef (Arthur, Brichta-Harhay, Bosilevac, Guerini, Kalchayanand, et al., 2008; Brichta-Harhay et al., 2012; Gragg et al., 2013). This has contributed to increased research and regulatory scrutiny of *Salmonella* and combined with the fact that illnesses due to *Salmonella* are not declining, the increased media, activist and legislator attention to the issue of foodborne salmonellosis could contribute to an FSIS decision to make some strains of *Salmonella* adulterants in meats. FSIS has announced that for all samples they currently test for STECs, they also will start testing for *Salmonella* in order to better understand what strains are present and at what levels. FSIS has been petitioned by the Center for Science in the Public Interest to make antibiotic resistant *Salmonella Heidelberg, Newport, Hadar and Typhimurium* strains adulterants in meat and poultry.

Several experimental pre-harvest interventions have been demonstrated to be effective for reducing the shedding of pathogens in cattle (Callaway, 2010). Although most of them have either not received regulatory approval, or not been found as efficacious in large scale commercial trials. The most promising pre-harvest intervention strategies include sodium chlorate, probiotics, vaccines, and bacteriophages. Although primarily used for their effects on animal efficiency, direct-fed microbials (probiotics) have the most widespread adoption. Supplementing livestock feed with microbial additives is becoming more common and is used to improve health, increase growth rate and efficiency and reduce foodborne pathogens. The application of bacteriophage to cattle hides at the processing plant during warm seasons has been adopted as well, but to a more limited

extent. Numerous other interventions are being studied and may eventually be proven useful enough for implementation (Callaway, 2010).

2. Antimicrobial interventions

Live animals and the environment serve as sources of pathogenic microorganisms which can contaminate carcasses during the slaughtering (harvest) process (from hide, paunch contents, and fecal material; Lahr, 1996) and meat products during processing, storage, and handling (processing tools and equipment and human contact). Animal products not only can become contaminated with microorganisms, but also support their growth if not properly handled, processed, preserved, and cooked which may result in a significant public health threat. Three issues in the production of meat products can have an important impact on the risk of contamination: 1) level of pathogens contaminating the hides of animals; 2) proficiency in hide removal that minimizes transfer of contamination from the hide to the carcass; and 3) efficacy of antimicrobial interventions applied at various steps in the process.

The level of pathogens on hides can be impacted by several factors. There is seasonal variation with pathogen levels generally higher in the warmer months (Barkocy-Gallagher et al., 2003). Effective pre-harvest interventions targeted at the animal directly or the production environment could reduce the level of hide contamination of animals delivered to the processing plant. However, contamination of hides in the lairage environment (Arthur, Bosilevac, Brichta-Harhay, Guerini, Kalchayanand, et al., 2007; Arthur, Bosilevac, et al., 2008) could negate pre-harvest intervention efforts unless industry-wide implementation occurred or effective lairage interventions were implemented. Application of an effective hide intervention could reduce hide contamination regardless of lairage contamination such as hide-on carcass washing (Arthur, Bosilevac, et al., 2008).

Proper training and emphasis on application of best practices for hide removal can significantly impact the level of carcass contamination. Numerous industry efforts to maintain proper techniques include best practices' documents, webinars, and workshops to assist with employee training (BIFSCO, 2009). Arthur et al. (2004) developed a sampling protocol to enable processors to monitor their process and detect when it was not in control. This sampling protocol provides a comprehensive appraisal of processing plant microbial hygiene at multiple points throughout the harvesting process. Implementation of this sampling protocol into the standard operating procedures of beef processing plants was a recommendation of the first *E. coli* O157:H7 Summit conducted by BIFSCO in 2003 and has been widely adopted.

Regardless of the above efforts, beef processors should assume that some level of carcass contamination will occur and implement a multi-hurdle strategy of decontamination interventions. Decontamination steps during the slaughtering process can reduce contamination and contribute to improvement of shelf-life (Huffman, 2002) and safety of meat. The USDA-FSIS has recognized that a decontamination step should be a part of the slaughtering/dressing process (USDA, 1996). To comply with regulatory criteria established by the USDA-FSIS (USDA, 1996), the beef industry focuses primarily on meat decontamination through application of various interventions (Bacon et al., 2000; Koohmaraie et al., 2005, 2007; Sofos & Smith, 1998). The decontamination strategies applied to fresh beef are intended to reduce levels of spoilage and pathogenic microorganisms.

Antimicrobial agents have long been studied for their effectiveness to inactivate or inhibit growth of microorganisms in and on foods (Huffman, 2002). The ability of the antimicrobials to be utilized in food and food products is described in the FSIS directive (USDA-FSIS, 2013). Antimicrobial agents can be classified into three categories: (a) processing aids, added to the food during processing and either removed or converted into normal food constituents or functional additives without leaving significant residuals, (b) secondary direct food additives, added during processing for functionality and removed from the final food products without technical effect from residuals and no required labeling, and (c) direct food additives, provide technical effects to the final food products and should be labeled by their common name. Antimicrobial treatments for improving food safety can be applied to meat products and are allowed to result in up to a 0.5% gain in weight.

2.1. Post-harvest interventions

The selection of antimicrobial interventions depends upon several factors such as desired effect, legal limits of use, cost, and effect on the food. Most of the interventions have been focused at the post-harvest phase because studies have shown that the hide is the primary source of carcass contamination (Arthur, Bosilevac, et al., 2008; Barkocy-Gallagher et al., 2003; Bell, 1997; Koohmaraie et al., 2005; Newton, Harrison, & Wauters, 1978; Nou et al., 2003; Small et al., 2005; Sofos et al., 1999), and that contamination is best removed immediately, before bacteria attach firmly to the meat surface (Anonymous, 2006). Hide contamination can come during skinning or hide pulling with potential, but less likely, sources including ruptured intestines. Another less frequent source is stomach contents flowing back out of the esophagus after head removal. In general, a multi-hurdle approach using washing and sanitizing compounds has been effective in reducing bacterial populations and the presence of pathogens on carcasses as long as the load does not exceed the capacity of the interventions (Koohmaraie et al., 2007). Numerous antimicrobial interventions have been used in the beef industry, but the following interventions are recognized as some of the most effective and/or promising tools used today as part of pathogen control and their general efficacies are summarized in Fig. 1.

2.1.1. Physical interventions

Microorganisms can be physically removed from a carcass in order to reduce the microbial level and make other antimicrobial steps that follow them more effective. Knife trimming, steam-vacuuming, and ambient temperature water washing are the most common physical interventions. In most cases, trimming of the affected product is an acceptable corrective action for visible contamination. The mean aerobic plate count was reported around 3 logs less than on carcasses where no trimming was carried out (Prasai et al., 1995). Greater reductions using both trimming and washing in combination than using either treatment alone were reported, but no combination resulted in the complete elimination of pathogens such as *E. coli* O157:H7, *Salmonella* or *Listeria* from the carcasses (Reagan et al., 1996). Steam-vacuuming has been widely implemented at multiple stages in processing and is effective for removing visible contamination (Dorsa, Cutter, & Siragusa, 1996, 1997). Steam-vacuuming is particularly effective for use on hide-removal pattern lines but not necessarily for the entire carcass surface (Dorsa et al., 1997).

2.1.2. Acid antimicrobials

Organic acids such as acetic, citric, and lactic acids are some of the more widely studied antimicrobial agents (Belk, 2001). The effectiveness of organic acids as antimicrobials differs widely based on concentration, pH, pKa, and the concentration of the undissociated molecule (Baird-Parker, 1980). The specific mode of action of organic acids as an antimicrobial is not known, but is likely a combination of actions of the undissociated molecules and the dissociated ions causing interference with transmembrane proton gradient of the microbial cells, and interference with three-dimensional structures of cell surface, outer membranes, and cytoplasmic membrane (Booth & Kroll, 1989; Brown & Booth, 1991; Corlett & Brown, 1980; Eklund, 1989). These changes can interfere with nutrient transport and energy generation and in turn interfere with microbial growth. In addition, low pH can reversibly and irreversibly damage cellular macromolecules that subsequently can inflict sublethal injury as well as lethal injury to microbial cells.

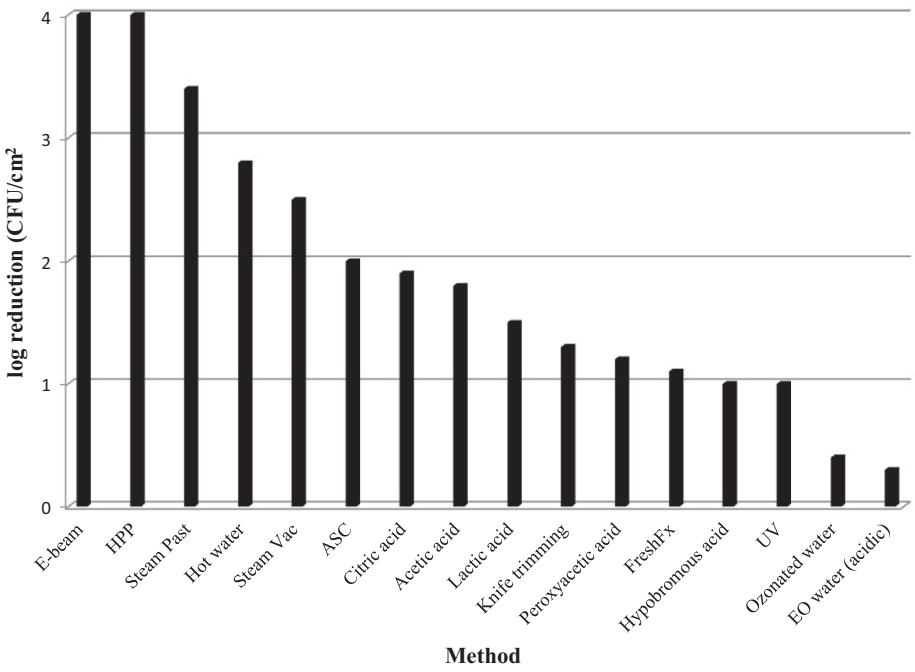

Fig. 1. Relative log reductions[a] for intervention methods[b] currently used or under investigation to reduce *E. coli* O157:H7 contaminated on surfaces of fresh meat.

Currently, most organic acids are allowed for use at 1.5 to 2.5% of the solution for pre-chilled carcass washing in commercial plants for both beef and lamb dressing (USDA-FSIS, 1996), although some can be used at levels up to 5% concentration. Organic acid treatment for 10 to 30 s resulted in 1 to 3 log microbial reduction (Dubal et al., 2004; Kalchayanand et al., 2008; Ramirez, Acuff, Lucia, & Savell, 2001; Ransom et al., 2003). The efficacy of acid treatment methods varies depending on the length of time the bacteria have in contact with the meat surface (Anonymous, 2006) and whether the bacteria are protected on the surface by fat, small cuts, or the uneven carcass surface, such that the organic acid is unable to come into contact with the cell. The temperature of the carcass surface, the presence of moisture, and solidification of fat surfaces during cooling are all likely to affect the ability of organic acid treatment to effectively decontaminate a carcass (Anonymous, 2006). Organic acid treatments have been shown to be most effective when applied as a warm (50 to 55 °C) carcass rinse (Acuff, 2005). Unfortunately, the corrosive effects of the organic acids on processing equipment increase as the temperature rises. Other limitations of organic acid treatment are possible discoloration of the lean, organoleptic changes, and development of acid-resistant pathogens.

Lactic acid is the most common organic acid used in meat industry for decontamination of products due to a combination of effectiveness and cost. Lactic acid (2%) was shown to reduce *E. coli* O157:H7 on beef carcass tissue by 3.3 logs, and 2% acetic acid reduced it by 1.6 log (Ransom et al., 2003). These authors also found that lactic acid and acetic acid treatments on cheek meat, using spray or immersion, resulted in 1.1 log reduction in total bacteria. The lesser reductions were attributed to the physical structure of cheek meat surfaces which may protect microbes from the treatments. Schmidt et al. (2014) demonstrated that 2% lactic acid was effective for reducing contamination on cheek meat when soaked for 1 min. Following a request from the European Commission, the Panel on Biological Hazards (BIOHAZ Panel) and the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) were asked by the European Food Safety Authority (EFSA) to deliver a Scientific Opinion on an application submitted by the USDA for the approval of lactic acid (concentrations from 2 to 5% (wt/wt) at temperatures of up to 55 °C applied by either spraying or misting) for uses to reduce microbial contamination of beef hides, carcasses, cuts and trimmings (EFSA, 2011). Lactic acid was approved by the European Union in February, 2013 for these purposes (Commission Regulation (EU), 2013). The effectiveness of lactic acid against STEC strains inoculated on surfaces of fresh beef including STEC O157:H7 and non-O157 STEC has been demonstrated (Geornaras et al., 2012; Kalchayanand, Arthur, Bosilevac, Wells, & Wheeler, 2013; Kalchayanand et al., 2012).

Reductions of *E. coli* O157:H7 on lean and adipose tissues of beef carcasses with intact fascia treated with 1%, 3%, or 5% acetic acid, lactic acid or citric acid were similar; acid type did not significantly influence observed pathogen inhibition (Cutter & Siragusa, 1994). Spray application for 20 s of the commercial antimicrobial compound Beefxide®, containing blended lactic and citric acids reduced the populations of *E. coli* O157:H7 and *Salmonella* by 1.4 and 1.1 log CFU/100 cm^2 on inoculated fresh beef (Laury et al., 2009). Spraying 2% FreshFX™ (Sterifx, Shreveport, LA), containing citric, phosphoric and hydrochloric acids, produced a 1.1 log reduction of *E. coli* O157:H7 on beef heads and cheek meat (Kalchayanand et al., 2008). Citrilow™ containing citric and hydrochloric acids effectively reduced aerobic plate counts, coliform and *E. coli* on inoculated fresh beef (Pohlman et al., 2010). Citrilow (2%) spray treatment reduced *E. coli* O157:H7, non-O157 STEC including O26, O45, O103, O111, O121, and O145 serogroups, and *Salmonella* on surfaces of fresh beef approximately 1.5 log (Kalchayanand, 2014). Various combination products have been implemented in commercial beef processing.

2.1.3. Oxidizer antimicrobials

Oxidative biocides are proposed to have multiple targets within a cell as well as in almost every biomolecule; these include peroxidation and disruption of membrane layers, oxidation of oxygen scavengers and thiol groups, enzyme inhibition, oxidation of nucleosides, impaired energy production, disruption of protein synthesis and, ultimately, cell death (Dean, Stocker, & Davies, 1997; Dodd, Sharman, Bloomfield, Gordon, & Stewart, 1997; Imlay, 2003). When a stronger oxidant is used, the electrons are transferred to the microorganism much faster, causing the microorganism to be deactivated rapidly.

Peracetic acid or peroxyacetic acid is approved by FSIS for use on beef carcasses (FDA, 2003a). Currently, the maximum allowance of peracetic acid for use in the meat industry is 400 ppm, but 200 ppm is normally used for washing, rinsing, cooling, or otherwise processing of fresh beef carcasses. A few studies have been reported for peracetic acid treatment to reduce *E. coli* O157:H7 load on meat carcasses (Gill & Badoni, 2004; Kalchayanand et al., 2012; King et al., 2005; Penney et al., 2007; Ransom et al., 2003; Stopforth et al., 2004). Under laboratory conditions, peracetic acid treatment produced a 1.0 to 1.4 log reduction of *E. coli* O157:H7 inoculated onto beef carcass tissue (Ransom et al., 2003). A study by King et al. (2005) noted that peracetic acid reduced *E. coli* O157:H7 and *Salmonella* Typhimurium by 0.7 logs on hot carcass surfaces. In contrast, beef and veal carcasses that were either sprayed with 180 ppm peracetic acid alone or water followed by peracetic acid resulted in mean log reductions of *E. coli* O157:H7 of 2.29 and 2.56, and 2.67 and 3.39, respectively (Penney et al., 2007). It is widely used in beef processing.

In recent years, electrolyzed oxidized (EO) water has gained attention as a disinfectant used in the food industry. EO water is produced by passing a current of electricity through a dilute saltwater solution. One product of the reaction is sodium hydroxide (NaOH), and the other is hypochlorous acid, which has a low pH, contains active chlorine, and has a strong oxidation reduction potential similar to that of ozone. EO water has been shown to reduce populations of *Listeria monocytogenes* (4.3 to 5.2 logs) and *Staphylococcus aureus* (1.7 to 1.9 logs) on stainless steel, and *Campylobacter jejuni* on poultry carcasses (4.9 logs; Kim, Hung, & Russell, 2005; Ayebah, Hung, & Frank, 2005; Park, Hung, & Kim, 2002). However, EO water reduced *E. coli* O157:H7 inoculated on surfaces of beef heads and cheek meat of less than 0.5 log CFU/cm^2 (Kalchayanand et al., 2008). Bosilevac et al. (2005) reported that EO water reduced total aerobic count on cattle hides by 3.5 logs, and *Enterobacteriaceae* counts by 0.9 log while reducing *E. coli* O157:H7 prevalence from 82% to 35%. Recent results of EO water experiments have shown that *E. coli* O157:H7 is more resistant that the non-O157 STEC to treatment and that the reductions of these organisms generally correlate with the increased levels of free chlorine in the EO water (Ravirajsinh, Hung, & Bosilevac, 2013).

Acidified sodium chlorite (ASC) is approved for use in the U.S. at concentrations between 500 and 1200 ppm. The antimicrobial activity of ASC is attributed to the oxidative effect of chlorous acid, which is derived from the conversion of chlorite ion into its acid form under acidic conditions such as mixing with citric or phosphoric acid. Several studies have demonstrated a 1.9 to 2.3 log reduction in *Salmonella* and *E. coli* O157:H7 on beef carcass tissue using a wash/spray of sodium chlorite activated (acidified) with citric acid (Ransom et al., 2003). One laboratory trial showed up to 4.6 log reductions in *E. coli* O157:H7 and *Salmonella* using a water wash followed by an ASC spray (Castillo, Lucia, Kemp, & Acuff, 1999). ASC reduced the top six non-O157 STEC inoculated on surfaces of fresh beef ranging from 0.6 to 2.0 log CFU/cm^2 (Kalchayanand et al., 2012). Other studies indicate limited success (Gill & Badoni, 2004). Some commercial implementation has occurred.

Ozone is a water-soluble, naturally occurring gas which is a powerful oxidizing agent. It destroys microorganisms by attacking and oxidizing the cellular walls and membranes. Ozone is very unstable, and on exposure to air and water it rapidly decomposes to form oxygen, hence, it must be generated at the point of use. Reductions of 2.5 logs have been reported on beef tissue using 0.5% ozonated water (Gorman, Sofos, Morgan, Schmidt, & Smith, 1995). Other researchers have reported reductions of 1.3 log or less with no difference between a water wash containing aqueous ozone applied to hot carcasses or heads compared to that of water alone (Castillo, McKenzie, Lucia, & Acuff, 2003; Kalchayanand et al., 2008; Reagan et al., 1996). In a study where ozonated water was used in a simulated hide washing system (Bosilevac et al., 2005), there was a reduction of 2.1 logs in the total aerobic count on the hides, compared with water alone, which only reduced the total microbial count by 0.5 log. The greatest drawback to the use of ozone is that the exposure limit for those in the immediate area of its use is 0.1 ppm (OSHA, 2011), thus rendering any experimentally identified concentration (0.5% = 5000 ppm) too hazardous to use in most commercial settings.

Hypobromous acid is an active antimicrobial agent, and has been utilized for a long time in processing water for can or bottle pasteurizers and coolers (Sun, Allen, Luckie, Wheatley, & Worley, 1995). In the poultry industry, hypobromous acid at a level of 100 ppm has been approved for use as an antimicrobial in chiller water during processing (FDA, 2003b). For the beef industry, hypobromous acid is allowed up to 900 ppm, but is most commonly used at 300 ppm for decontamination of carcasses. Hypobromous acid reduced aerobic plate counts and *Enterobacteriaceae* by 2.8 to 3.6 log CFU/cm^2, *E. coli* O157:H7 by 1.6 to 2.1 log CFU/cm^2, and *Salmonella* by 0.7 to 2.3 log CFU/cm^2 on fresh beef and beef hearts, respectively (Kalchayanand et al., 2009). In contrast, soaking cheek meat into a solution of hypobromous acid for 1 min was not effective for reducing STEC or *Salmonella* (Schmidt et al., 2014). Hypobromous acid was commonly used at various stages of beef processing until late 2013 when it was omitted from the Pathogen Reducing Technologies listed in the FSIS Export Library for Japan (USDA-FSIS, 2014).

2.1.4. Thermal interventions

The main objective of heat treatment is to destroy vegetative cells and spores of microorganisms including molds, yeasts, bacteria, and viruses (Olson & Nottingham, 1980). Steam-vacuuming is approved for use by USDA-FSIS as a substitute for knife trimmings to remove visible fecal and ingesta contamination and is widely adopted in beef processing. Steam-vacuuming is only useful for application to specific areas of the carcass that are known to be heavily contaminated and for spot treatment of visible contamination i.e. it is not feasible to 'steam-vacuum' an entire carcass. The equipment is a hand-held device consisting of a vacuum wand with a hot spray nozzle, delivering water at 88 to 94 °C to the carcass surface under pressure, while simultaneously vacuuming the area (Dorsa, 1996; Dorsa et al., 1996); thus, it is a combination of physical and thermal treatments. Steam-vacuuming reduced the aerobic plate count, total coliform count, *E. coli* count, and *E. coli* O157:H7 count by 3.0, 4.0, 4.0, and 5.5 logs CFU/cm^2, respectively on inoculated beef short plates (Dorsa, 1996; Dorsa et al., 1996, 1997). Other studies that used two different hot water/steam-vacuum systems found that aerobic plate counts and total coliform counts to be reduced by 1.1 to 2.3 logs and 1.2 to 2.2 logs, respectively (Kochevar, Sofos, Bolin, Reagan, & Smith, 1997). Some bleaching of the carcass surface occurred using the system, but was not a permanent discoloration. Steam-vacuum use after chilling failed to remove inoculated *Salmonella* possibly because the organisms had been allowed the time during chilling to become firmly attached to the surface and form biofilms (Bacon, Sofos, Belk, & Smith, 2002).

Hot water as an intervention step has been extensively researched and automated cabinet designs are widely used around the world. The mode of action of heat treatment is mainly by inactivating the most sensitive vital enzymes (denaturation) for bacterial life as well as causing DNA strand breakage and RNA degradation (Ray, 2001). Barkate, Acuff, Lucia, and Hale (1993) reported that a 95 °C spray for 10 s raises the carcass surface temperature to 82 °C. Sprays of 95 °C for 5 s at 24 psi reduced up to 3 logs in total coliforms, thermotolerant coliforms, *S.* Typhimurium, and *E. coli* O157:H7 (Huffman, 2002), but maintaining such a high delivery temperature is difficult and expensive, especially at the volumes required by commercial processing plants. Hot water (85 °C) for 15 s at 15 psi reduced *E. coli* O157:H7 and top six non-O157 STEC between 3.2 and 4.2 log CFU/cm^2 on inoculated surfaces of fresh beef (Kalchayanand et al., 2012). Spraying at high pressure may not achieve the desired temperatures at the contact surface, and may generate condensate and aerosol. Low pressure spraying would give higher tissue temperatures than high pressure, as it allows for a longer contact time, but high pressure will promote greater physical

removal of contamination. Automated hot water wash cabinets for pre-evisceration and final carcass interventions are common in U.S. beef processing plants.

Steam pasteurization is based on the fact that steam at 100 °C has a much higher heat capacity than water at the same temperature, so if steam condenses on a surface, the temperature of that surface rises more rapidly than if it were water that was deposited on the surface. Furthermore, steam droplets are small and can penetrate and inactivate the bacteria in the cavities on the surface (Morgan, Radewonuk, & Scullen, 1996). Significant reductions have been demonstrated in total aerobic plate count and *E. coli* counts on beef carcasses from steam pasteurization (Dorsa et al., 1996; Nutsch et al., 1998). A commercial trial showed significant reductions in *E. coli* and *Enterobacteriaceae* at sites where initial numbers were high, but it did not result in complete elimination of these bacteria (Minihan, Whyte, O'Mahoney, & Collins, 2003). Steam pasteurization significantly reduced *E. coli* O157:H7, *S. Typhimurium*, and *L. monocytogenes* counts by 3.5, 3.7, and 3.4 log CFU/cm^2, respectively, on surfaces of inoculated pre-rigor beef (Phebus et al., 1997). Steam treatment to raise surface temperatures of pre-rigor beef to as low as 71 °C for 6 s reduced *E. coli* O157:H7, non-O157 STEC serogroups O26 and O111, and *Salmonella* counts by approximately 3 log CFU/cm^2 (Kalchayanand, 2014). Automated steam pasteurization cabinets for final carcass interventions are another common feature in U.S. beef processing plants.

Hot water and steam thermal treatments can cause a cooked/bleached appearance, depending on the treatment time and temperature, but the discoloration is usually less noticeable after a few hours of chilling (Castillo, Hardin, Acuff, & Dickson, 2002). However, because of the effectiveness of thermal treatments, industry and customers have accepted some level of surface heat denaturation and a thermal treatment of either hot water or steam is used at most beef processing plants.

2.1.5. Non-thermal interventions

Although thermal treatment is the most effective in killing/inactivating microbiological contaminants, thermal processing can induce physical and chemical changes in food. Non-thermal processing technologies are alternative interventions that use little heat to reduce microbial contamination while minimizing the quality and nutrient losses. Some non-thermal technologies such as electron beam, ultraviolet (UV) light and UV-ozone combination, cold atmospheric plasma, and high pressure processing are being used or investigated as interventions in the beef industry.

Electron beam (E-beam) irradiation uses a stream of high-energy electrons, known as beta rays, which can penetrate only about 15 mm, while X-irradiation has intermediate penetration (Zhu, Du, Cordray, & Ahn, 2005). Mode of action of irradiation involves damaging the bacterial cells' genetic material and disrupting their normal functions. The biggest obstacle to irradiation as an intervention is consumer acceptance. There is a perception that irradiation is dangerous to health, which in large doses, it is, but the doses required to treat foods are tiny and considered to be safe after many years of research. Doses of 1.0 to 10.0 kGy have been shown to be effective in food decontamination, while 0.4–0.6 kGy would give a 1 log reduction in *L. monocytogenes* (Radomyski, Murano, & Olson, 1994). Low-dose/low-penetration E-Beam irradiation has now evolved to the point where large non-uniform surface areas can be effectively treated, which allows whole carcasses to be treated after chilling. Only the surface (about 15 mm penetration) receives a significant radiation dose (Koohmaraie et al., 2005). It has been shown that a 1 kGy dose of E-beam radiation applied to chilled beef primals reduced *E. coli* O157:H7 numbers by 4 log cycles, with no adverse effects on the sensory attributes of the meat, as judged by a trained taste panel (Arthur et al., 2005). Use on beef carcasses has been awaiting the outcome of an FSIS petition to consider E-beam a processing aid not requiring irradiation labeling. A limited number of companies use it on ground beef.

Ultraviolet (UV) light irradiation is commonly used in hospitals and laboratories for decontamination of surfaces, air and water. UV treatment has been used for a number of years in water purification and research is ongoing into the application of UV directly to foods (Chun, Kim, Lee, Yu, & Song, 2010; Sommers, Cooke, Fan, & Sites, 2009). UV is an electromagnetic wave, lying outside the band of visible light. The effective wavelength for bactericidal activity is at 253.7 nm and at certain wavelengths (180 nm) produce ozone, which enhances the antibacterial effect (Kaess & Weidermann, 1973). UV light causes permanent cross-links to form in the microbial DNA, preventing the cell from carrying out its normal functions (Sastry, Datta, & Worobo, 2000). Lately, UV and ozone treatments have received attention from the beef processing industry because it is a non-thermal processing technology that does not leave any chemical residues on products or cause any physical damage (Khadre, Yousef, & Kim, 2001). The successes of UV light treatment have been reported against *Salmonella* on poultry (Wallner-Pendleton, Sumner, Froning, & Stetson, 1994) and against *Pseudomonas aeruginosa* (Abshire & Dunton, 1981). Most studies have used low intensity UV for 9 min or more, but if high intensity UV light was used, exposure times could be less than 10 s (Stermer, Lasater-Smith, & Brasington, 1987). UV irradiation for 75 s resulted in approximately 1.0 log reduction for *E. coli* O157:H7 and six regulated non-O157 STEC, while treatment with UV-ozone combination for 75 s resulted in an additional 0.2 log reduction of these pathogens inoculated on fresh beef (Kalchayanand, Bosilevac, & Wheeler, 2013). Some commercial use of UV on beef products has been implemented, but not for ozone.

Cold atmospheric plasma (CAP) is a weak ionized gas that operates below 40 °C at the point of application. The plasma can be produced in air or inert gas to generate reactive molecules like atoms, ions, and radicals (Stoffels, Sakiyama, & Graves, 2008). These reactive molecules cause damage to microbial spore coats and cell wall materials, with fatal outcomes (Laroussi, 2005). Early work on ionization of air showed that the surface of meat could be decontaminated using this kind of technology, and there have been claims of 80% reductions in microbial load on carcasses (Gysin, 1986) and that growth was inhibited, resulting in a 1 log difference during storage of beef or pork (Mackey & Mead, 1990). Recently, stable electron fields have been established as outlined above, and researchers have been able to inactivate cultures of *E. coli* in times ranging from 4.5 s to 5 min (Maeda, Igura, Shimoda, & Hayakawa, 2003). This technology has not yet been commercially implemented.

High pressure processing (HPP) is another non-thermal method of combating microbial contamination of food products. The advantage of HPP is that it destroys microorganisms throughout the entire food product, and when appropriately used, HPP does not alter the texture, appearance or flavor of foods. HPP involves packaged food placed in the pressure vessel and submitted to water pressures from 100 to 1000 MPa (Kalchayanand, Sikes, Dunne, & Ray, 1998). Mode of action of hydrostatic pressure to microorganism cells is not fully understood, but high pressure damages cellular membranes, resulting leakage of cell contents, oligomeric proteins and protein complexes also undergo dissociation (Gross & Jaenicke, 1994). HPP-treated *L. monocytogenes* had reduced gene expression and the effect was dependent on the intensity and time of the treatment (Bowman, Bittencourt, & Ross, 2008). HPP reduced by more than 5 log cycle populations of *E. coli* O157:H7 (Gola, Mutti, Manganelli, Squarcina, & Rovere, 2000), *Pseudomonas fluorescens*, *Citrobacter freundii* and *Listeria innocua* in ground beef (Carlez, Rosec, Richard, & Cheftel, 1993). Microbial reductions are enhanced when high pressure treatment is combined with mild heating or chilling, but color changes were observed after 10 min of treatment. The use of pulsed high pressure can be more effective than continuous single application, so treatment times can be reduced (Hayakawa, Kanno, Yoshiyama, & Fujio, 1994). HPP treatment of fresh ground product has been targeted to use in hotel and restaurant markets, but has not been used for retail markets due to the negative effects on product color.

2.1.6. Multi-hurdle strategy

No single intervention is 100% effective. There is natural variation among bacteria in their susceptibility to any one intervention type. In addition, the uneven beef carcass surface provides many opportunities for pathogens to avoid contact with interventions. Many studies have shown that using combinations of interventions throughout the process (the multiple-hurdle approach) gives greater microbial reductions than using any single intervention (Bacon et al., 2000; Sofos, 2005). The use of two or more food safety technologies in a sequence may achieve a synergistic effect, or at least an additive effect. Arthur et al. (2004) demonstrated that by minimizing deposition of bacteria onto the carcass and using subsequent effective food safety technologies, processors can maintain *E. coli* O157 populations below detectable levels on all of the carcasses tested after chilling. Hardin, Acuff, Lucia, Oman, and Savell (1995), using beef primals, found that a wash with 35 °C water, followed by a rinse with acetic or lactic acid is more effective than single treatments of knife trimming or water washing at reducing inoculated levels of *S.* Typhimurium and *E. coli* O157:H7. Bacon et al. (2000) also reported that when carcasses moved through multiple stages of treatment, aerobic plate counts reduced from 6.1–9.1 log CFU/100 cm^2 to 3.8–7.1 log CFU/100 cm^2 and *E. coli* counts reduced from 2.6–5.3 log CFU/100 cm^2 to 1.0–3.0 log CFU/100 cm^2, respectively. A multi-hurdle approach provides insurance against the variation in contamination coming in on the hides and minimizes the chance that the hide load and subsequent carcass load will exceed the capacity of the interventions.

The intervention technologies mentioned above are published in scientific literature available to support the validation procedures. Any attempt to determine the optimal food safety technology, solely based on reductions reported in the scientific literature should be approached with caution, and validation of the method under actual in-plant conditions will ultimately be necessary and is required by FSIS. Many of the published studies are carried out under controlled or laboratory conditions, and therefore, the effect must be demonstrated to be similar in commercial application.

2.1.7. Conclusions

Post-harvest interventions have greatly improved the safety of beef. Numerous antimicrobial intervention compounds have been researched and implemented for carcasses, as well as strategies such as steam vacuuming, hot water washing, steam pasteurization, and hide-on carcass washing systems. Understanding and training on best carcass dressing procedures have improved greatly. A multi-hurdle approach is commonly used to maximize the reduction in risk of pathogen contamination.

2.2. Pre-harvest interventions

Although post-harvest interventions have been extremely effective, *E. coli* O157:H7 positive tests from FSIS sampling and industry test-and-hold sampling still occur. Furthermore, HEP positives seemingly represent considerable risk of subsequent foodborne illnesses. Thus, sporadic failures in process control or incoming pathogen load exceeding intervention capacity could be reasons for positive pathogen tests. The goal of implementing effective pre-harvest interventions would appear to have multiple benefits. If pathogen loads on hides of cattle presented for slaughter were reduced, this should reduce the risk of exceeding post-harvest intervention capacity. This appears particularly important considering the concept that supershedder cattle may contribute a majority of hide pathogen loads after lairage environment effects (Arthur, Brichta-Harhay, et al., 2010; Arthur et al., 2009). Reduced pathogen shedding also reduces potential environmental contamination.

A prerequisite for successful pre-harvest intervention implementation is adherence to basic production best practices of maintaining clean feed, clean water, a well maintained environment and appropriate biosecurity related to pests (BIFSCO, 2013). Although it is difficult to demonstrate that these activities alone will lead to reduced pathogen shedding, the implementation of these good animal-health management principles sets the foundation for successful pre-harvest interventions.

It is unlikely that a pre-harvest intervention will provide a 100% effective strategy. However, if cost-effective approaches are identified that can be used in a multi-hurdle approach as has been successful in post-harvest interventions, then perhaps meaningful reduction in pathogen shedding that results in reduced pathogen load on hides presented for slaughter could be achieved. If supershedder cattle are contributing a majority of the contamination of hides, then the requirement of a pre-harvest intervention strategy is not elimination of shedding but elimination or near elimination of supershedders. Another requirement would be industry-wide adoption or other means to overcome the animal-to-animal cross contamination that occurs in the lairage environment.

2.2.1. Sodium chlorate

Sodium chlorate has been demonstrated to be effective in reducing shedding in cattle when added to feed or water. Chlorate is reduced to chlorite which kills bacteria (Stewart, 1988). In vitro and in vivo studies appear to confirm the efficacy of sodium chlorate as a feed or water treatment in several species for reducing the levels of pathogens shed in the feces (Anderson et al., 2000, 2001, 2002; Callaway et al., 2002, 2003) and in levels on the hide (Anderson et al., 2005). The use of chlorate to reduce foodborne pathogenic bacteria in food animals is presently and has been under review by the U. S. Food and Drug Administration for many years, it has not been approved at this time (Callaway, 2010).

2.2.2. Probiotics

Direct-fed microbials have been used in the livestock industry to improve production efficiency (Martin & Nisbet, 1992). More recently they have been studied for their potential to reduce the level of pathogen shedding, although, few have demonstrated consistent results (Arthur, Bosilevac, Kalchayanand, Wells, Shackelford, et al., 2010). Although several commercial products are available, a *Lactobacillius*-based product appears to be the most effective for reducing *E. coli* O157:H7 shedding in cattle (Brashears, Jaroni, & Trimble, 2003). Use of Bovamine® and Bovamine Defend® has been growing. Bovamine® has been demonstrated to have beneficial effects on feed efficiency and average daily gain. The higher level of bacteria in Bovamine Defend® (10^9 *Lactobacillus acidophilus* NP51 + *Propionibacterium freudenreichii* NP24) is purported to combine improved growth efficiency that may cover the cost of the product with food safety benefits of reduced pathogen shedding, although it has no FDA approved label claim for food safety.

2.2.3. Vaccines

Two *E. coli* O157:H7 vaccines have been widely studied for reduction of fecal shedding. The Epitopix SRP® vaccine is conditionally approved in the U.S. and targets siderophore proteins and disrupts iron transport into the bacterium which kills the cell. Preliminary research generated promising results (Fox et al., 2009; Thornton et al., 2009), but large-scale commercial trials did not support the cost of the product. Econiche® is an *E. coli* O157 bacterial extract vaccine also with promising preliminary data (Moxley et al., 2009; Smith, Moxley, Klopfenstein, & Erickson, 2009; Van Donkersgoed, Hancock, Rogan, & Potter, 2005), but inconsistent cost/benefit outcomes in large commercial trials. Econiche® is fully licensed in Canada but not licensed in the U.S. and pursuit of full license with FDA in the U.S. may be discontinued. A *Salmonella* Newport vaccine is used primarily in the dairy industry, but cull dairy cow slaughter facilities may additionally benefit from reduced *Salmonella* contamination from its use.

2.2.4. Bacteriophages

Viruses that kill bacteria (bacteriophages) occur naturally in the environment. Because of their generally narrow target of specific surface receptors on bacteria, they have the advantage of specificity of pathogens of interest without wide-spread microbial ecology disruption. However, this also may be a disadvantage due to rapid development of resistance to specific phages, thus, cocktails of phages may be necessary with frequent rotation of specific phages. FSIS has approved phage treatment of cattle hides in the holding pens at beef processors before slaughter for reduction of *E. coli* O157:H7 contamination. Two commercial products, Finalyse® from Elanco and another from Omnilytics, use cocktails of lytic phages for a hide wash application. Elanco research concludes that a significant reduction in *E. coli* O157:H7 positive trim is obtained when cattle are sprayed with the product before slaughter. The application of bacteriophage to cattle hides after unloading at the processing plant has been implemented on a limited scale by at least one processor during the warm seasons of the year, but little data are available to verify effectiveness.

2.2.5. Conclusions

It is generally agreed that pre-harvest interventions may be needed to make significant additional progress. This is especially true if the hypothesis that "supershedder animals" are contributing the majority of the pathogens that eventually contaminate beef products is correct. However, effective pre-harvest approaches have proved much harder to identify and implement and are further complicated by slow regulatory approval, difficulty finding an economic model to facilitate their implementation, and the problem of the lairage environment contaminating all animals upon arrival at the processing plant. Feed and water additives such as sodium chlorate and probiotics, as well as vaccines and bacteriophages show some of the greatest potential as pre-harvest interventions. Direct-fed microbials have had the most widespread adoption due to their effects on efficiency, but some products are gaining in use as evidence increases for potential food safety effects. The application of bacteriophage during warm seasons to cattle hides after unloading at the processing plant has been implemented on a limited scale but little data are available to verify effectiveness. Vaccines have been developed for *E. coli* O157:H7 and for *Salmonella* but have limited use in beef production. Sodium chlorate appears effective in experimental trials, but has not been approved by FDA. It is possible that moderately effective technologies could significantly impact food safety by being effective enough to eliminate or dramatically reduce occurrence of supershedders, but this makes a difficult cost/benefit argument for the segment of the industry that implements the technology.

3. Summary

Food safety systems today use the multi-hurdle approach that may include pre-harvest interventions, such as probiotics and bacteriophage, combined with post-harvest interventions such as hide-on carcass wash, steam vacuuming, knife trimming, pre-evisceration wash, final carcass wash with various compounds, thermal treatment with hot water or steam, spray chill with antimicrobials, chilled carcass spray before grading, subprimal, trim, and ground beef treatments. *Salmonella*, *E. coli* O157:H7 and currently the top six non-O157 STEC are the main targets of carcass pathogen-reduction programs. Current discussions regarding which pathogens should be regulated focus on their pathogenicity rather than just serotypes. FSIS has started collecting baseline data on *Salmonella* in beef products and it is likely that some kind of *Salmonella* regulation will occur within 1 to 2 years. Several promising non-thermal interventions are being evaluated and could contribute to improved safety in the future while reducing water usage and quality damage. Other strategies also contribute to the food safety system such as non-competitive food safety information sharing, emphasis on best practice carcass dressing procedures and test-and-hold programs. Exciting future developments that will likely enhance the safety of meat include validation of non-thermal antimicrobial interventions, whole genome sequencing that will lead to improved detection of pathogens, and the ability to develop accurate, rapid tests for pathogen strains demonstrated to be the highest risk to cause human illnesses.

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