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Effect of Fecal Contamination and Cross-Contamination on Numbers of Coliform, *Escherichia coli, Campylobacter,* and *Salmonella* on Immersion-Chilled Broiler Carcasses†

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ABSTRACT

The effect of prechill fecal contamination on numbers of bacteria on immersion-chilled carcasses was tested in each of three replicate trials. For each trial, 16 eviscerated broiler carcasses were split into 32 halves and assigned to one of two groups. Cecal contents (0.1 g inoculated with *Campylobacter* and nalidixic acid–resistant *Salmonella*) were applied to each of eight halves in one group (direct contamination) that were placed into one paddle chiller (contaminated), whereas the other paired halves were placed into another chiller (control). From the second group of eight split birds, one of each paired half was placed in the contaminated chiller (to determine cross-contamination) and the other half was placed in the control chiller. Postchill carcass halves were sampled by a 1-min rinse in sterile water, which was collected and cultured. Bacterial counts were reported as log CFU per milliliter of rinsate. There were no significant statistical differences (paired *t* test, $P < 0.05$) from direct contamination for coliforms (mean 3.0 log CFU) and *Escherichia coli* (mean 2.7 log CFU), although *Campylobacter* numbers significantly increased from control values because of direct contamination (1.5 versus 2.1 log CFU), and the incidence increased from 79 to 100%. There was no significant effect of cross-contamination on coliform (mean 2.9 log CFU) or *E. coli* (mean 2.6 log CFU) numbers. Nevertheless, *Campylobacter* levels were significantly higher after exposure to cross-contamination (1.6 versus 2.0 log CFU), and the incidence of this bacterium increased from 75 to 100%. *Salmonella*-positive halves increased from 0 to 42% postchill because of direct contamination and from 0 to 25% as a result of cross-contamination after chilling. Water samples and surface swabs taken postchill from the contaminated chiller were higher for *Campylobacter* than those taken from the control chiller. Immersion chilling equilibrated bacterial numbers between contaminated and control halves subjected to either direct contamination or cross-contamination for coliforms and *E. coli*. *Campylobacter* numbers, *Campylobacter* incidence, and *Salmonella* incidence increased because of both direct contamination and cross-contamination in the chiller. Postchill *E. coli* numbers did not indicate which carcass halves were contaminated with feces before chilling.

Immersion chilling is the traditional and preferred method of chilling for processed broiler carcasses in the United States. Benefits of immersion chilling include relatively efficient heat transfer, maintenance of the appearance of product per consumer expectations, and reported decrease in bacteria numbers *(2, 4, 8, 9, 16, 19, 20–22, 24, 31, 34).* Problems with immersion chilling include the use of large quantities of potable water, the cost of cleaning water before discharge, and possible increased risk of cross-contamination. Potentially, one or a few ''dirty'' carcasses could cross-contaminate many ''clean'' carcasses through close contact with carcasses or water in the chiller. The Food Safety Inspection Service (FSIS) has decided that overall product safety, whether impaired from direct contamination before the chiller or from cross-contamination in the chiller, can be improved by mandating zero tolerance for visible fecal contamination of carcasses entering the chiller *(35).*

Methods developed to reduce numbers of bacteria and subsequent cross-contamination include washing carcasses with inside and outside bird washers (IOBW) before chilling, adding freshwater inflow during chilling, using continuous counterflow systems instead of parallel-flow systems, and adding chlorine or other antimicrobials to the chiller water. One study found IOBWs were minimally effective at reducing *Campylobacter* contamination on broiler carcasses *(2).* Washing might not improve food safety *(13),* yet IOBWs have greatly increased water usage by the poultry industry. Rate of freshwater addition to the chiller had little effect on *Salmonella* numbers *(33),* but overall higher use of water appeared to decrease coliform numbers *(5).* The use of freshwater inflow increases water usage and costs, however. Continuous-flow chilling was found to be better than static chilling for reducing total numbers of bacteria and fecal streptococci *(12).* Counterflow immersion chillers, with clean water inflow at the end of the process so that exiting carcasses constantly move toward the cleanest water, were better for reducing bacterial numbers and incidence than parallel-flow chillers, in which clean water enters the same end of the chiller as the carcasses *(19).* Chlorine usage in chiller water decreases coliforms *(5), Campylobacter (37),* and *Salmonella (33, 37)* in the water.

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† Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

However, carcasses treated with chlorine are not suitable for export to several countries, and problems with chlorination persist.

Studies of cross-contamination occurring during immersion chilling have shown an increased incidence of carcasses positive for *Campylobacter (19)* and *Salmonella (16).* Another study demonstrated that *Salmonella* cross-contamination occurred during chilling, and although addition of extra chlorine decreased contamination, it still was not prevented completely *(33).* Although cross-contamination is possible, reports show that overall bacterial numbers and incidence levels tend to decrease with immersion chilling. Chilling can reduce the numbers of total bacteria *(9, 16, 20–22, 34),* coliforms *(4, 8, 19, 24), Enterobacteriaceae (9, 16, 21, 34), Escherichia coli (8, 16, 19, 21), Campylobacter (2, 31),* and psychrotrophs *(20, 22).* Evidence also exists that chilling reduces the incidence of *Campylobacter (2, 14, 31)* and *Salmonella (23, 25).*

Although FSIS has mandated a zero-tolerance policy for fecal material on carcasses before chilling, the success of this policy on improving food safety has been questioned *(13).* A previous study reported that presence of visible feces before the chiller was not indicative of *Salmonella* incidence on postchill carcasses *(17). Salmonella* incidence actually increased on postchill carcasses that were not visibly contaminated with feces, either because of cross-contamination in the chiller or normal variation between carcasses. A previous research report demonstrated that halved carcasses provided an appropriate method for reducing sample variation compared with the variation present between two or more different carcasses *(7).* Therefore, the objective of this experiment was to determine whether immersion chilling, isolated from other factors known to decrease bacterial numbers or incidence, affects the microbiology of broiler carcass halves subjected to either direct contamination or cross-contamination with feces.

MATERIALS AND METHODS

Three replicate trials were conducted, each with 16 broiler carcasses obtained from a local processing plant just after the final IOBW and before the chiller. At the plant, carcasses were individually bagged on removal from the line and were transported to the laboratory. Carcasses were aseptically removed from bags and cut in half vertically along the midline with a sterile knife *(7).* Both halves were tagged for identification.

Approximately 20 broiler intestinal tracts were obtained from the same plant at the same time as carcass collection and placed together in a clean plastic bag. Intestines were separated and cecal contents were removed and pooled. Contents were stirred manually with a sanitized spatula *(3).* On a per gram basis, *Campylobacter* (10^7 CFU), an isolate from broiler chicken feces, and a nalidixic acid–resistant *Salmonella* Typhimurium (10^6 CFU) were added to the cecal contents. The mixture was again manually stirred to thoroughly mix the cultures with the cecal material. Samples of this mixture were taken and coliforms, *E. coli, Campylobacter,* and *Salmonella* numbers were determined as described below.

To simulate fecal contamination, 0.1 g of the cecal content mixture was applied to the breast area of each of eight chicken halves. The other eight paired halves remained untreated to serve as controls. All paired carcass halves were left uncovered at room temperature for 10 min to simulate the maximum time carcasses would typically be exposed in a plant to fecal contamination resulting from evisceration (approximating the time from the beginning of evisceration to final bird washing). An additional eight paired halves were left untreated and placed uncovered in the room for 10 min.

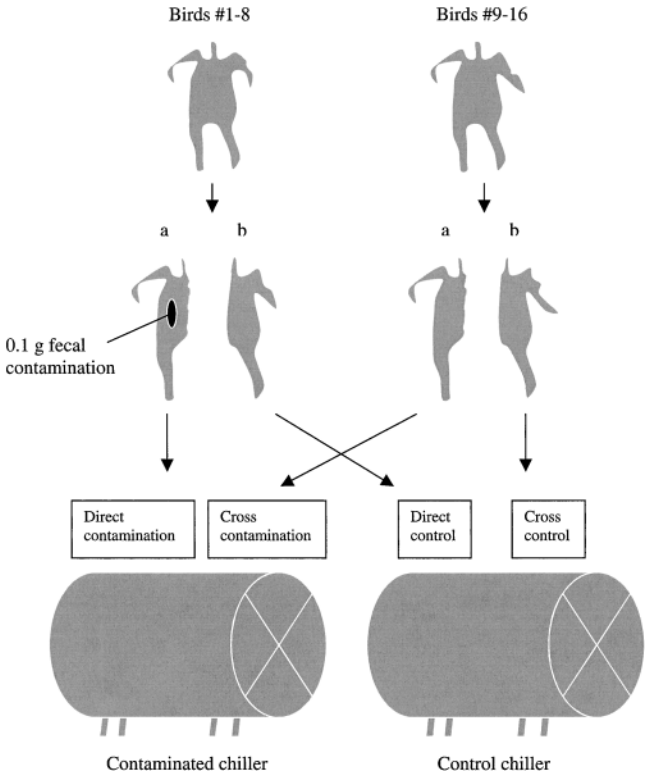

FIGURE 1. *Bird assignments by number and half to direct contamination or cross-contamination treatments and contaminated or control chillers.*

Two similar prototype paddle chillers were prepared by filling with approximately 150 liters of water and ice slush (0.3°C). For each trial, one sponge (Nasco, Ft. Atkinson, Wis.) premoistened with 10 ml of phosphate-buffered saline (PBS) was used to swab an inner surface of approximately 200 cm^2 in each chiller before addition of carcasses. PBS (10 ml) was added to the sponge and manually compressed for 30 s to mix, and the liquid was removed for 10-fold serial dilutions and plating on media as described below. Also for each trial, one water sample was taken from each filled chiller with a sterile specimen cup, transferred to sterile tubes, and centrifuged for 10 min at 1,600 $\times$ *g.* The pellet was resuspended in 10 ml of PBS, which was used for 10-fold serial dilutions and plating on media as described below.

Chicken halves treated with feces were placed in one chiller, without any washing or removal of feces (contaminated), whereas the untreated paired half was placed in the other chiller (control). The other eight untreated paired halves were divided, by pair, and added into each of the chillers (see Fig. 1). Twelve additional uncontaminated whole carcasses were added to each chiller *(8)* to approximate industry chiller loading standards of 7.5 liters per bird. Chillers were operated for 45 min, with an endpoint internal carcass half-temperature of approximately 2.0°C. No inflow of freshwater was used, and no chlorine was added. After chilling, the carcass halves were removed and another surface swab and water sample was taken from each chiller.

Carcass halves were subjected to a low-volume whole-car-

TABLE 1. *Means ± SEM (log CFU per milliliter of rinsate) and incidence of total coliforms,* E. coli, Campylobacter, *and* Salmonella *from postchill broiler carcass halves from the contaminated chiller and postchill halves from the control chiller* [a]

Organism	Contamination treatment	Bacteria (log CFU/ml rinsate)		Probability
		Contaminated	Control	
Coliforms	Direct	3.0 ± 0.1 (24/24)	3.0 ± 0.1 (24/24)	0.2760
	Cross	2.9 ± 0.1 (24/24)	2.9 ± 0.1 (24/24)	0.1462
E. coli	Direct	2.7 ± 0.1 (24/24)	2.6 ± 0.1 (24/24)	0.4188
	Cross	2.6 ± 0.1 (24/24)	2.6 ± 0.1 (24/24)	0.2084
Campylobacter	Direct	2.1 ± 0.1 (24/24)	1.5 ± 0.1 (19/24)	<0.0001
	Cross	2.0 ± 0.1 (24/24)	1.5 ± 0.1 (18/24)	<0.0001
Salmonella	Direct	0.8 ± 0.1 (10/24)	ND (0/24)	—
	Cross	0.7 ± 0.1 (6/24)	ND (0/24)	—

[a] Direct, directly contaminated with 0.1 g cecal contents inoculated with *Campylobacter* and *Salmonella* and applied prechill; Cross, stored in the same chiller with the contaminated halves but not directly contaminated with cecal contents prechill; ND, not detected.

cass rinse procedure *(10).* Carcasses were rinsed by adding 100 ml of PBS to each carcass half in a bag and shaking with an automated carcass shaking machine for 60 s *(11).* Rinsates were aseptically collected from all carcass halves, and serial dilutions were prepared in PBS. *Campylobacter* was cultured by direct plating onto the surface of Campy-Cefex agar *(32),* which was incubated at 42°C for 48 h in a microaerophilic atmosphere consisting of 5% O_2, 10% CO_2, and the balance N_2 (BOC Gases, Chattanooga, Tenn.). Colonies with the characteristic appearance of *Campylobacter* were counted. Each colony type from every sample was confirmed as *Campylobacter* by observation of cellular morphology and motility on a wet mount with phase contrast microscopy. Each colony type was further confirmed by a positive reaction from a serological latex agglutination test kit (Panbio, Inc., Columbia, Md.). Coliform and *E. coli* were enumerated by plating 1 ml from a serial dilution of the sample onto duplicate petrifilm *E. coli* and coliform count plates (3M Health Care, St. Paul, Minn.). Petrifilm plates were incubated at 35°C for 18 to 24 h, and the types of colonies characteristic of coliforms and *E. coli* were counted. Serial dilutions were also plated onto the surfaces of BG-Sulfa agar (Becton Dickinson, Sparks, Md.) with the addition of 200 ppm sodium salt of nalidixic acid (Sigma Chemical Co., St. Louis, Mo.; BGS-NAL) for enumeration of nalidixic acid–resistant *Salmonella.* BGS-NAL plates were incubated at 35°C for 24 h, and colonies characteristic of *Salmonella* were counted.

Bacterial numbers were converted to log CFU for statistical analysis. Differences between carcass halves attributable to trial, contamination treatment, or chiller were tested by analysis of variance with SAS GLM procedures *(29).* Means were pooled across trials because no significant ($P < 0.05$) trial by treatment or chiller interactions were observed. Carcass halves without detectable numbers were treated as missing values. The paired *t* test in SAS *(29)* was used to determine differences between treated (direct or cross-contamination) and untreated control paired halves to show differences resulting from direct contamination or cross-contamination of feces on carcass halves.

RESULTS

The inoculated cecal contents (CFU/g) over the three trials contained from 7.2 to 8.0 log coliforms, 6.8 to 7.7 log *E. coli,* 6.9 to 8.0 log *Campylobacter,* and 5.8 to 6.1 log *Salmonella* (nalidixic acid–resistant). One-tenth gram of this mixture was placed on each contaminated carcass half, resulting in mean levels of approximately 6.5, 6.2, 6.6, and 5.0 log CFU/g coliforms, *E. coli*, *Campylobacter,* and *Salmonella,* respectively, per carcass half.

Mean log numbers of bacteria on carcass halves are shown in Table 1. The direct contamination treatment resulted in no significant ($P < 0.05$) differences between control halves in numbers of coliforms (3.0 versus 3.0 log CFU/ml rinsate) or *E. coli* (2.7 versus 2.6 log CFU/ml). Direct contamination significantly increased numbers of *Campylobacter* compared with control halves (2.1 versus 1.5 log CFU/ml). Nalidixic acid–resistant *Salmonella* did remain on directly contaminated carcass halves after chilling (0.8 log CFU/ml), demonstrating its ability to adhere to carcasses.

Carcass halves exposed to cross-contamination were not significantly ($P < 0.05$) different from control halves for mean numbers of coliforms (2.9 log CFU/ml rinsate) or *E. coli* (2.6 log CFU/ml). Cross-contamination resulted in higher numbers of *Campylobacter* than found on control halves (2.0 versus 1.5 log CFU/ml). Cross-contaminated carcass halves did show mean numbers of nalidixic acid–resistant *Salmonella* postchill at 0.7 log CFU/ml.

Within the contaminated chiller, there were no significant ($P < 0.05$) differences in numbers of coliforms, *E. coli*, *Campylobacter*, or *Salmonella* between halves subjected to either direct contamination or cross-contamination. Similarly, there were no differences within the control chiller for untreated halves (randomly assigned contralateral control halves from the direct contamination and cross-contamination treatments) for coliforms, *E. coli,* or *Campylobacter.* No nalidixic acid–resistant *Salmonella* were detected on the halves in the control chiller.

Incidence of bacteria is also shown in Table 1. Direct contamination, for halves in the contaminated chiller, resulted in 24 of 24 positives for coliforms, *E. coli,* and *Campylobacter.* Ten of 24 halves in the contaminated chiller were positive for nalidixic acid–resistant *Salmonella* treated with direct contamination. Halves exposed to cross-contamination in the contaminated chiller had the same incidence (24 of 24) as direct contamination, except for *Salmonella,* for which only 6 of 24 halves were positive.

Halves in the control chiller used for paired controls

of the direct contamination and cross-contamination treatments were 100% positive (24 of 24) for coliforms and *E.coli.* For control halves, 19 of 24 and 18 of 24 halves were positive for *Campylobacter.* No nalidixic acid–resistant *Salmonella* was detected in the control chiller.

Chiller water samples taken from both chillers before addition of carcass halves contained no coliforms, *E. coli, Campylobacter,* or *Salmonella.* Postchill samples from the contaminated chiller had coliform, *E. coli, Campylobacter,* and *Salmonella* levels of 3.2, 2.7, 2.9, and 1.5 log CFU, respectively. The control chiller postchill samples had levels of coliforms at 3.1 log CFU, *E. coli* at 2.7 log CFU, and *Campylobacter* at 1.2 log CFU; no *Salmonella* was detected. Postchill coliform and *E. coli* means were calculated from two samples instead of three because of a dilution error.

Chiller surface swabs from both chillers before addition of carcass halves had no detectable coliforms, *E. coli, Campylobacter,* or *Salmonella* (data not shown). Postchill, contaminated chiller coliform numbers were 2.4 log CFU, *E. coli* 1.7 log CFU, and *Campylobacter* 1.7 log CFU, but no nalidixic acid–resistant *Salmonella* was detected. Postchill control chiller surfaces had 1.5 and 1.2 log CFU of coliform and *E. coli,* respectively. No *Campylobacter* or nalidixic acid–resistant *Salmonella* was detected in these samples. Two samples were averaged for postchill coliform and *E. coli* means instead of three because of a dilution error.

DISCUSSION

Neither direct contamination nor cross-contamination had any significant effect on coliform or *E. coli* numbers compared with the controls. This indicates that the background numbers of these organisms are already relatively consistent at the commercial plant of origin and that immersion chilling is capable of reducing higher numbers (when introduced as direct contamination) on carcasses to some level that appears constant. The significant increase in *Campylobacter* numbers for both direct contamination and cross-contamination shows that chilling alone is not sufficient to reduce this organism when introduced in high numbers or when control numbers are relatively low. Low numbers of *Salmonella* were recovered from direct-contaminated carcasses, indicative of the lower numbers added in the cecal content mixture. Although the numbers were low, there were sufficient bacteria to spread to the cross-contaminated carcasses.

Immersion chilling has been shown to decrease numbers of bacteria on broiler carcasses *(2, 4, 8, 9, 16, 19–22, 24, 31, 34).* In this experiment, prechill rinses were not conducted; however, from postchill control data and the numbers of bacteria added to directly contaminated carcasses, it can be deduced that immersion chilling reduced the number of bacteria isolated in this experiment. This theoretical reduction would be approximately 2 to 3 log units of inoculated bacteria as a result of immersion chilling. Reports cited previously typically found a reduction of closer to 0.5 to 1.5 log units of native or resident bacteria.

In a previous experiment, direct contamination of broiler carcass halves with 0.1 g of cecal contents, without washing or chilling, yielded 4.7 log coliforms, 4.4 log *E. coli,* and 5.6 log *Campylobacter* per carcass half *(3).* In a similar experiment, broiler carcass halves were inoculated with 0.1 g of cecal contents but were then spray washed and chilled. Numbers of coliforms and *E. coli* on postchill carcasses were reported to be 5.8 and 5.5 log CFU, respectively. Two of the three trials were negative for *Campylobacter (8).* This and two previous studies all were different with regard to the numbers of bacteria detected on carcass halves, probably because of a variation in numbers of residual bacteria on carcasses when acquired from the commercial processing plant. This variation in numbers and of incidence was one reason, along with ensuring their presence, for inoculating cecal contents with *Campylobacter* and *Salmonella.*

Within each chiller, bacterial numbers on halves seemed to equilibrate to within approximately 0.1 log unit for each of the bacteria enumerated, suggesting a relatively stable environment for transfer of cells among carcasses. Previous research shows that including chlorine in the chiller *(18, 33)* and the use of counterflow chillers rather than parallel-flow chillers *(19, 28)* can lower bacterial numbers. However, another researcher reported chlorine added to chiller water did not reduce numbers of *Campylobacter* and *Salmonella* attached to carcass skin *(37).* All halves were positive for the presence of coliforms and *E. coli* regardless of contamination treatment or chiller used. Neither direct contamination nor cross-contamination had an effect on the incidence of either coliforms or *E. coli* because all halves were probably positive at the beginning of the study. Chilling also did not lower the incidence of either coliforms or *E. coli.* Both direct contamination and cross-contamination resulted in all halves testing positive for *Campylobacter* (24 of 24 positive) compared with control halves, in which 19 of 24 and 18 of 24, respectively, were positive in the control chiller. Because all 24 halves were inoculated with *Salmonella* before chilling, the chilling process lowered *Salmonella* incidence by 58% (14 of 24) for those halves that were contaminated with feces. However, 25% (6 of 24) of those halves exposed to cross-contamination were found to be positive for *Salmonella.*

Our data agree with the results of previous researchers who reported that immersion chilling reduced coliform numbers but gave no information regarding incidence. Therefore, it could be assumed that there was no change in incidence in those studies *(1, 4, 5, 8).* In a direct comparison, no difference in the incidence of coliforms or *E. coli* was shown to be the result of chilling *(27).* Parallel-flow chilling slightly increased the incidence of *E. coli,* whereas counterflow chilling slightly decreased the incidence of this organism on postchill carcasses *(19).* Before inoculation, most of the halves were apparently positive for *Campylobacter* or the chiller was able to remove enough bacteria from the control halves (with lower starting numbers) to result in a lower incidence compared with the direct contamination or cross-contamination treatment halves. In this experiment, there was a 21 to 25% difference in *Campylobacter* incidence as a result of chilling between the control

and contaminated carcass halves. Other researchers have shown that *Campylobacter* incidence on chicken carcasses typically decreases by 20% *(2),* 16% *(14),* and 14% *(31)* with immersion chilling.

The reduction on contaminated carcasses but increase on carcasses exposed to cross-contamination could explain the mixed results from previous studies, which have shown that *Salmonella* incidence can increase *(16, 21, 26)* or decrease *(23, 25)* with chilling. The importance of chlorine is apparent in a report that demonstrated that *Salmonella* decreased in incidence as chlorine levels increased *(33).*

Negative results on prechill water show that potable water and initial surfaces were clean before use. Presence of bacteria in postchill water from both chillers show that bacteria are washed from carcasses during the chilling process and, because no chlorine was added, survive in the water. Similar numbers were observed between chiller water samples for coliforms and *E. coli,* with slightly less *Campylobacter* and no *Salmonella* in the control chiller. Therefore, it appears that the addition of feces to carcasses placed directly in the chiller had little effect on water quality, even without the use of chlorine. The following bacteria have been isolated from water samples taken from commercial poultry plant chillers: total bacteria *(4, 21, 26), Enterobacteriaceae (21),* coliforms *(1, 4), E. coli (19), Campylobacter (19, 30),* and *Salmonella (19, 21).* Variability has been reported for bacterial numbers in chiller water samples by location in the chiller *(1, 4, 26)* and by type of chiller, in which counterflow chiller samples had lower numbers than parallel-flow chiller water samples *(19, 28).* Chlorine was not used in this study, so the effect of immersion chilling alone on numbers and incidence of bacteria could be determined. The addition of chlorine would likely have reduced or eliminated bacteria in water samples, as has been reported previously *(36, 37).* Without chlorine or other treatments, there were ample bacteria present to allow cross-contamination during chilling.

Both chiller surfaces were clean before chilling because no bacteria were detected. The control chiller numbers were lower than the contaminated chiller numbers, so postchill chiller surfaces seem to be sensitive to the initial level of contamination. Little research has been conducted on the microbiology of chiller surfaces in chicken processing plants. One report found *Campylobacter* contamination of approximately 1 to 2 log units on the chiller exit trough in three chicken processing plants during operation *(15).* Another report found no evidence of *Salmonella* contamination on equipment surfaces at the chiller exit either before startup or after the operational shift in a commercial turkey processing plant *(6).* Surfaces can become contaminated and act as a source of possible cross-contamination. Current results and previous reports demonstrate the importance of maintaining the proper sanitation of equipment surfaces in plants.

Immersion chilling, even under adverse conditions (no prechill spray wash, no freshwater inflow, no continuous or counterflow current, and no added chlorine), is effective at reducing numbers of bacteria, including pathogens, on carcasses. Even with the extreme conditions in the contaminated chiller (40% of carcasses with large areas of visible feces), coliform and *E. coli* numbers and incidence levels were not increased by either direct contamination or cross-contamination compared with the control chiller. *Campylobacter* and *Salmonella* numbers also decreased from the calculated load placed on carcass halves through direct contamination. However, immersion chilling alone is insufficient to prevent cross-contamination of carcasses by *Campylobacter* and *Salmonella.* Chlorine or other antimicrobials should be incorporated into the chiller water because they can reduce cross-contamination by killing bacteria suspended in the water. In a previous experiment, postchill bacterial numbers of carcass halves with feces applied, then spray washed before chilling, showed no increase in coliforms or *E. coli (8).* In this study, we also demonstrates that unsprayed carcasses contaminated with visible feces were indistinguishable from controls after immersion chilling with regard to coliform and *E. coli* numbers.

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