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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Coliforms, *Escherichia coli*, *Campylobacter*, and *Salmonella* in a Counterflow Poultry Scalder with a Dip Tank[1]

J.A. Cason[2] and A. Hinton, Jr.
U. S. Department of Agriculture, Agricultural Research Service,
Russell Research Center, P. O. Box 5677, Athens, Georgia 30604-5677, USA

Abstract: Suspended bacteria were enumerated in scald water and carcass rinse samples from a commercial broiler chicken processing plant with a multiple-tank, counterflow scalder. Coliforms, *Escherichia coli*, and *Campylobacter* were enumerated and the Most Probable Number (MPN) of salmonellae was determined in water samples from each of three scald tanks, from a dip tank located between defeathering machines, and in rinses of carcasses removed from the processing line immediately after defeathering. Mean coliform concentrations in Tanks 1, 2, and 3 were 4.6, 2.5, and 1.6 $\log_{10}$(cfu/ml), respectively. *E. coli* concentrations followed the same pattern with means of 4.4, 2.1, and 1.4 in Tanks 1, 2, and 3, respectively, with significant differences ($P<.05$) in the concentrations of both coliforms and *E. coli* between tanks. Mean *Campylobacter* concentration in four positive samples from Tank 1 was 4.0 $\log_{10}$ (cfu/ml), but only one water sample from Tank 2 and none from Tank 3 were *Campylobacter* positive. Coliforms and *E. coli* were found in dip tank samples in only two instances, with no isolations of *Campylobacter* or salmonellae. Mean numbers of coliforms, *E. coli*, and *Campylobacter* in carcass rinses were 3.1, 2.7, and 3.3 $\log_{10}$(cfu/ml). Salmonellae were isolated from five of six water samples from Tank 1 with a mean MPN of 13.3/100mL, but were isolated from only three of six water samples from Tank 2 and two of six from Tank 3. Salmonellae were isolated from half (18/36) of all carcass rinses. Most bacteria suspended in scald water were found in the first tank, with no *Campylobacter* or salmonellae found in the dip tank. Counterflow, multiple-tank scalders appear to reduce the opportunity for cross-contamination during scalding.

Key words: *Escherichia coli, Salmonella*, scalding, water, suspended bacteria

Introduction

Numbers of viable bacteria in poultry scald water reach a plateau after 20 to 60 min of operation, depending on temperature and other conditions (Mercuri *et al*., 1974; Mead, 1989), although the plateau may drift upward as scald water pH changes (Humphrey and Lanning, 1987). A plateau is implied in scalder models (Veerkamp, 1989; Veerkamp *et al*., 1991; Cason and Shackelford, 1999) and is seen in sampling data from scald tanks in the first 2 hours of operation (Veerkamp *et al*., 1991). Many bacteria have D values (time of exposure to produce a one log or 90% reduction in numbers of bacteria) in the 5 to 20 min range for water conditions in a typical industrial scald tank. In 20 minutes of operation, 2800 carcasses will pass through a scald tank in a plant running 140 birds per minute, so the number of bacteria in a single scald water sample will be influenced by the numbers of bacteria coming from hundreds of carcasses, or thousands of carcasses if the strain has a relatively high D value. The variability in counts between individual carcasses going into the tank has little impact on numbers of suspended bacteria, especially if the scalder has been operating long enough to reach a plateau.

A literature search found no reports of microbiological sampling at different points within a single industrial scald tank, but sampling of water at multiple points within a model tank to which food coloring was added at a single location indicated that concentration in all samples moved rapidly toward equilibrium (Cason and Shackelford, 1999). The tank in that study had a single line of simulated carcasses passing through the tank, so water should be well mixed in multiple-pass industrial scalders where water can mix between the lines of carcasses.

Despite widespread introduction of multiple-tank, counterflow scalders into poultry processing plants in the last 15 years, there are relatively few reports of numbers of bacteria suspended in water in multiple-tank scalders. Veerkamp and Heemskerk (1992) reported that lower numbers of *Enterobacteriaceae* were recovered in water samples from successive tanks in a three-tank industrial scalder. Decreasing amounts of suspended solids and numbers of aerobic bacteria (Cason *et al*., 1999b) and decreasing numbers of coliforms and *E. coli* (Cason *et al*., 2000) have been reported in water samples from successive tanks, based on sampling of a three-tank scalder with the same volume and temperature in all tanks.

Although the design and placement of scalding and defeathering equipment is strongly influenced by the limited space available in most processing plants,

scalder design and operation have continued to evolve. The average length of time that carcasses are scalded appears to have increased somewhat compared to a few years ago, and there may be more variation in scald tank size and operating temperatures than when multiple-tank scalders were first being installed in plants. Another innovation in some plants is the use of a dip tank or short scald tank located between defeathering machines.

Although cross-contamination of bacteria occurs during scalding (Mulder *et al.*, 1978), sampling of processed carcasses indicates that multiple-tank, counterflow scalders and other technological changes can reduce some bacterial counts on the final carcass (James *et al.,* 1992; Waldroup *et al.*, 1992, 1993). This paper reports numbers of coliforms, *E. coli*, *Campylobacter*, and incidence of salmonellae in water samples and carcass rinses obtained in a commercial processing plant with a three-tank, counterflow scalder and a short dip tank located between the second and third defeathering machines. The three scald tanks contained different volumes of water maintained at different temperatures, with a total scald time of almost 4 min, in contrast to earlier reports which sampled three-tank scalders with identical volumes maintained at the same temperature with a scald time of 2 min (Cason *et al.*, 1999b; Cason *et al.*, 2000).

Materials and Methods

In the plant chosen for sampling, the counterflow scalder consisted of three multiple-pass tanks. Tank 1 was a 4-pass tank with carcasses exiting near where they entered, and Tanks 2 and 3 were three-pass tanks that carcasses entered and exited on opposite ends. Scald water mixed between the lines of carcasses within each tank. Clean water was added to Tank 3 and flowed by gravity into the other tanks, with overflow from Tank 1. After two defeathering machines, there was a short dip tank followed by a final defeathering machine.

Line speed was 140 carcasses per min with carcasses spaced every 15.2 cm. Residence time was approximately 90, 90, and 55 s in Tanks 1, 2, and 3, respectively. Tank capacity was approximately 30 L per carcass with overflow of approximately 1 L per carcass. Residence time in the one-pass dip tank was approximately 7 s.

Scald water samples of 200 mL each were removed from each tank in the scalder on six different days over a period of two months. Samples were taken while the plant was in operation after five-week old broilers had been processed for approximately 8 h. Water temperature was recorded for each tank with an electronic probe. Scald water samples of approximately 200 mL were removed at the first turn-around in each tank and were held in crushed iced until samples were analyzed within 2 h.

Immediately after water samples were taken, 6 carcasses were aseptically removed from the processing line after defeathering and before entering a carcass washer. Carcasses were placed in plastic bags and transported to the laboratory in a cooler with crushed ice. At the laboratory, all carcasses were sampled within 1 h. After removal of feet, 400 mL of 0.1% peptone water[3] was added to each bag. Carcasses were rinsed in an automated shaker for 1 min, after which carcasses were removed from the bags and discarded. The rinse liquid was poured into sterile plastic containers which were refrigerated at 4°C until microbiological analysis was performed within 1 h.

Microbiological analysis: Coliforms and *E. coli* were enumerated by adding 1 mL of serial dilutions of samples to Coliform/*E. coli* Petrifilm™[4] that was incubated at 35°C for 24 h. *Campylobacter* were enumerated on *Campylobacter* agar (Blaser)[5]. Inoculated *Campylobacter* plates were incubated 48 h at 42°C under microaerobic conditions produced with an activated BBL CampyPak Plus gas generator envelope in a BBL Gas-Pak Jar System. *Campylobacter*-like colonies were confirmed with the Latex-CAMPY culture confirmation test.[5]

A five-tube Most Probable Number (MPN) assay was performed to enumerate salmonellae in water samples. Serial dilutions of scald water samples were added to Rappaport-Vasiliadis Broth[6] (RV) and incubated overnight at 42°C. The following day 1 mL from each incubated tube was transferred to a fresh tube of RV broth and incubated at 42°C overnight. The contents of each tube were then streaked on XLT4 and Brilliant Green Sulfa agar incubated at 35°C overnight. Suspect colonies were inoculated into LIA and TSI slants incubated at 35°C overnight. Positive results were confirmed with poly-O antiserum, and all other tubes or plates were recorded as negative. Numbers of salmonellae in each sample were determined with a standard MPN table (Health Canada, 2005). A total of 55.55 mL was enriched from each scald water sample. Carcass rinse samples were tested for incidence of salmonellae by incubating 30 mL of the rinse liquid overnight at 37°C. Aliquots of 0.1 mL and 0.5 mL were transferred into 10 mL of RV broth and TT broth, respectively, followed by overnight incubation at 42°C. The contents of each tube were then streaked on XLT4 and Brilliant Green Sulfa agar incubated at 35°C overnight, after which the isolation procedure followed the MPN method described above.

Statistical Analysis: Estimates of numbers of bacteria were converted to $\log_{10}$ (cfu/volume) before statistical analysis. Numbers of bacteria in scald water samples were analyzed by PROC GLM of SAS® (SAS Institute, 2000) in a random block design to compare

Table 1: Coliforms, *E. coli*, and *Campylobacter* (log_{10} cfu/mL) recovered from scald water in Tanks 1, 2, and 3 in a multiple-tank, counterflow scalder in a commercial processing plant after 8 h of operation (140 carcasses/min) and from six defeathered carcasses removed from the line at the same time and sampled by whole carcass rinse (mean ± s.d.)

	Scald tank			Carcass rinses	
Bacteria	1	2	3	n	mean
Coliforms	4.6±0.3[a]	2.5±0.5[b]	1.6±0.5[c]	36	3.1±0.4
E. coli	4.4±0.4[a]	2.1±0.9[b]	1.4±0.4[c]	36	2.7±0.4
Campylobacter[d]	4.0±0.3	1.9	ND	24	3.3±0.7

[abc] Within a tank, means without common superscripts are significantly different ($P<0.05$). [d]In six water samples taken from each tank, *Campylobacter* spp. were isolated four times from Tank 1, one time from Tank 2, and were not detected in Tank 3 or the dip tank.

Table 2: Salmonellae (MPN/100 mL) recovered from scald water in a multiple-tank, counterflow scalder in a commercial processing plant after 8 h of operation (140 carcasses/min) and incidence of salmonellae on defeathered carcasses removed from the line at the same time and sampled by whole carcass rinse

	Scald tank				Carcasses
Day	1[a]	2	3	Dip tank	+/ total
1	6.1	2.0	<1.8*	<1.8	2/6
2	2.0	<1.8	<1.8	<1.8	3/6
3	7.5	<1.8	2.0	<1.8	3/6
4	17.0	7.8	<1.8	<1.8	3/6
5	<1.8	<1.8	<1.8	<1.8	2/6
6	34.0	2.0	2.0	<1.8	5/6
Mean	<11.4	<2.9	<1.9	<1.8	(18/36)

[a]Tank 1 is the first tank that carcasses enter. Clean water is added to Tank 3. Carcasses passed through two defeathering machines before entering the dip tank and passed through one additional defeathering machine before whole carcasses were removed from the line for whole carcass rinses. *<1.8 is the MPN table value when no salmonellae-positive tubes are found.

concentrations of bacteria in the different tanks. Day of sampling was blocked to focus on differences between tanks and remove variation due to day of sampling from the analysis. Separation of means was done with orthogonal contrasts.

Results and Discussion

The processing plant was operating under typical conditions on all days that samples were taken. Mean scald water temperatures were 51.9, 55.1, and 55.9°C in Tanks 1, 2, and 3, respectively. The mean dip tank temperature was 62.2°C.

Coliforms, *E. coli*, and *Campylobacter* recovered in scald water samples are shown in Table 1. Numbers of coliforms and *E. coli* suspended in tank water declined significantly from Tank 1 to Tank 2 and again from Tank 2 to Tank 3. The same pattern of declining numbers of bacteria during multiple-tank scalding has been reported previously (Veerkamp and Heemskerk, 1992; Cason *et al.*, 1999b; Cason *et al.*, 2000). The two-log reduction in coliforms and *E. coli* between Tank 1 and Tank 2 means that there were 100 times fewer bacteria suspended in the water in Tank 2 than in Tank 1. There was a further one-log reduction in coliforms and *E. coli* from Tank 2 to Tank 3. *Campylobacter* were isolated from four of the six samples taken from Tank 1, from one sample from Tank 2, and were not isolated in water samples from Tank 3. This result indicates that the pattern for the other bacteria was repeated with *Campylobacter*. Samples from the dip tank contained the fewest bacteria with one coliform isolation, one *E. coli*, and no *Campylobacter* isolations in the six samples taken from that location. The temperature of the dip tank was high enough to kill almost all bacteria of interest.

Numbers of coliforms, *E. coli*, and *Campylobacter* recovered in whole carcass rinses are also shown in Table 1, and are typical of reports in the literature for carcasses sampled immediately after removal of feathers (Cason *et al.*, 1999b, 2000). Substantial numbers of bacteria were found on defeathered carcasses despite declining numbers in successive scald tanks and the near absence of any bacteria in the dip tank after most feathers had been removed. In an experiment where carcasses were defeathered between the tanks of a three-tank scalder, there were no differences in *E. coli* and *Campylobacter* counts in rinse samples as compared to carcasses that completed scalding before any feathers were removed (Cason *et al.*, 1999a), so use of a dip tank may have no effect on bacteria in carcass rinses. All scald water samples and carcass rinses were negative for *Campylobacter* on the second and fifth sampling days when the flocks being processed appeared to be *Campylobacter* negative. On the other four sampling days, *Campylobacter* bacteria were isolated from all 24 carcasses sampled, with a mean of 3.3 logs per mL of rinse, so the failure to isolate *Campylobacter* from water in Tank 3 did not indicate that the carcasses passing through the tank did not carry the bacteria.

Isolations of salmonellae from scald tanks and carcass rinse samples are shown in Table 2. Salmonellae were isolated from the 6 scald water samples on 5, 3, and 2 occasions in Tanks 1, 2, and 3, respectively, and were never isolated from the dip tank. The declining trend of salmonellae isolations in successive scald tanks agrees with the results in Cason *et al.* (2000). The arithmetic mean of all salmonellae-positive samples from Tank 1 was 13.3 MPN/100 mL. Humphrey and Lanning (1987) reported an MPN of 13.9 salmonellae

per 100 mL of scald tank water in a tank operated at 50.5°C. Cason *et al.* (2000) reported a similar mean MPN of 10.9 salmonellae per 100 mL of scald tank water in positive samples from the first tank of a multiple-tank scalder operating with a water temperature of 55.8°C. The high incidence of salmonellae found in carcass rinses (18 of 36 carcasses) shows that low incidence in the final scald tank does not indicate that carcasses are free of salmonellae. Consistent reports of sharply declining numbers of bacteria suspended in the water in successive tanks of multiple-tank scalders indicate that opportunities for cross-contamination during scalding are probably being minimized by the multiple-tank design, as compared to single-tank designs.

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

[2]Author for correspondence: john.cason@ars.usda.gov

[3]Unless specified otherwise, microbiological media were obtained from Becton Dickinson, Sparks, MD 21152.

[4]3M Health Care, St. Paul, MN.

[5]Integrated Diagnostics, Inc., Baltimore, MD 21227.

[6]Oxoid Ltd., Basingstoke, Hants., England.