

REDUCTIONS OF *ESCHERICHIA COLI*, COLIFORMS, AEROBIC PLATE COUNTS AND *CAMPYLOBACTER JEJUNI* BY A SMALL-SCALE, HIGH-PRESSURE SYSTEM DEvised TO CLEAN A MINIATURIZED POULTRY GIBLETS TRANSPORT SYSTEM

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ABSTRACT

The efficacy of using direct high-pressure hot water (60C, 140F) and a quaternary ammonium compound to clean the inside of stainless steel pipe used to transport chicken giblets was evaluated. The giblets were collected from a commercial processing plant and were inoculated with Campylobacter jejuni. The cleaning system was effective in reducing the numbers of inoculated C. jejuni and naturally occurring mesotrophic bacteria (aerobic plate counts) on the inside surface of the stainless steel pipe used to transport the giblets. However, the decreases in naturally occurring Escherichia coli and coliforms were not significant. These results suggest that additional improvements are needed to better disinfect the piping system used to transport giblets to reduce the potential for cross-contamination with C. jejuni and E. coli. The devised cleaning system could be optimized to reduce the use of chemical agents, the cleaning time and the cost of cleaning pipes in poultry processing facilities.

PRACTICAL APPLICATIONS

These experiments suggest that the traditional use of hot water and quaternary ammonium compounds to clean the inside of the piping system

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used to transport chicken giblets may not be sufficient to reduce the contamination with *Campylobacter jejuni* and mesotrophic bacteria (aerobic plate count). Poultry processors should be aware of the limitations of cleaning closed piping systems and develop and test high-pressure systems to thoroughly clean the pipes used to transport giblets after processing to avoid potential sources of cross-contamination with *C. jejuni* and mesotrophic bacteria.

INTRODUCTION

In commercial broiler processing facilities, the hearts are usually collected after evisceration and are transported to tanks with cold water designed for the cooling of giblets (gizzard, heart and lungs of poultry). The gizzards are also collected after evisceration and are opened and the interior lining of the organ is removed with the remaining feed contents. The gizzards are usually harvested and processed independently from the heart and lungs, but comingle with the hearts in the same cooling tank and can be transported in the same piping system as the hearts. In poultry slaughter facilities, giblets must be chilled to 4C or below within 2 h of slaughtering the birds (Anon 2006). Currently, poultry giblets are sold for the production of pet foods and to less extent for human consumption (without lungs), to make gravy and appetizers or main dishes by people of fewer resources in the southern states of the U.S.A. or in developing countries (Anon 2006).

There are no clear figures associating giblets consumption with food safety risks. However, *Campylobacter* spp. and *Salmonella* can be commonly isolated from the viscera of different food production animals. For instance, *Salmonella* serotype Enteritidis has been associated with human disease by the consumption of improperly cooked poultry giblets (Anon 1984; Plummer *et al.* 1995; Rodrigo *et al.* 2006). In the case of *Campylobacter*, few articles have discussed the location of this organism in chicken giblets. It appears that the liver is the main organ contaminated with *Campylobacter* and when the giblets are transported using the same piping system, the chances of the liver contaminating the other organs are very high. The liver is an organ that can be easily broken during handling and transportation.

In Egypt, chicken hearts, livers and spleens were contaminated with *Campylobacter jejuni* with incidences of 28, 10 and 40%, respectively (Khalafalla 1990). *Campylobacter* spp. have been isolated from the surface and the interior of chicken livers sampled in the U.S.A. and New Zealand (Barot *et al.* 1983; Rodrigo *et al.* 2006). Because *Campylobacter* can also be found in deep tissues of porcine livers, gallbladders and bile ducts (Moore and Madden

1998), this may be a possible route of liver contamination in chickens. In New Zealand, more than 30% of the livers from broiler chickens have been reported to be positive for *Campylobacter* and carry more than 10^3 cfu per liver (Whyte *et al.* 2006).

In broiler processing facilities in the U.S.A., giblets are not regularly tested for the presence of *Campylobacter*. However, *Campylobacter* contamination may pose a similar risk as the contamination of poultry meat for people that consume giblets regularly. In addition, *C. jejuni* and *Campylobacter coli* have been isolated from the internal tissue of livers from pigs (Moore and Madden 1998) and chickens (Whyte *et al.* 2006). The packaging of broiler carcasses with or without giblets does not appear to influence the contamination rate of carcasses because no significant differences in contamination frequency were observed between carcasses wrapped with or without giblets in England (Anon 2003).

In poultry processing facilities, giblets are transported from the production area to the packing area in a large piping system. There may be several hundred feet of piping involved in the transportation process, some of which may run along the ceiling of the plant. Through a grant awarded by the Higher Education Challenge Grants Program of the U.S. Department of Agriculture for interdisciplinary studies and training of undergraduate students (Gale *et al.* 2005), we conducted experiments to determine the cleaning efficacy of a high-pressure device designed to clean a miniaturized transport giblets system. Because of the practical and economical limitations of testing an actual piping system, we designed a miniaturized piping system that was coupled to a pump that is commonly used in these processing operations. We performed a cleaning-in-place procedure during the experiments to determine the efficacy of the cleaning phases (hot water and quat). We present here the effects on bacterial reductions of a cleaning device used on a miniaturized system designed to transport giblets. The numerical reduction of naturally occurring mesotrophic bacteria (aerobic plate counts [APC]), *Escherichia coli* and coliforms, and inoculated *C. jejuni* on giblets are discussed based on the efficacy of a devised cleaning system.

MATERIALS AND METHODS

Design of the Transport System

A miniaturized transport system was made with 76 mm (3 in.) diameter sanitary stainless steel pipe type 304 (Stainless and Alloy Piping, Marietta, GA). The system included four 90° bends to create areas with more difficult access for complete cleaning. The piping system was attached to a Diaphragm

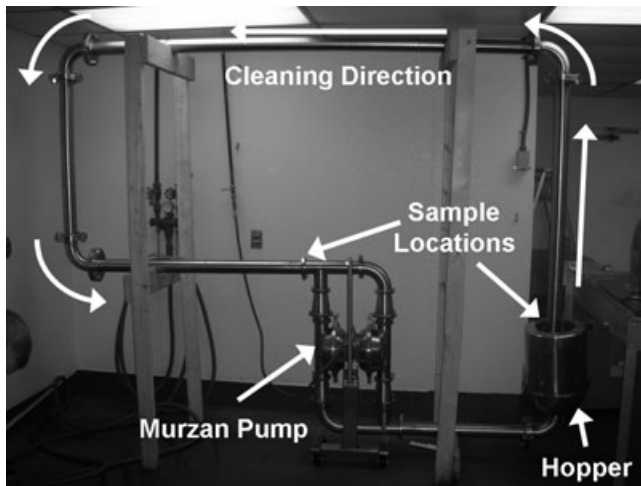


FIG. 1. PICTURE SHOWING THE PIPING SYSTEM DESIGNED TO TRANSPORT THE GIBLETS

Poultry Pump (Murzan, Inc., Norcross, GA), which was used to circulate the giblets through the piping system (Fig. 1).

Design of the Cleaning System

The general approach was to deliver high-pressure hot water to the inside of the steel pipe. The cleaning was accomplished using a rotary nozzle National Pipe Thread 9.5 mm (3/8 inch), 22 mm (7/8 inch) diameter, 58 mm (2.3 in.) length (Aqua Mole Technologies, Middleburg Heights, OH) with jets at 45° and 90° (relative to the axis of the pipe) that rotated around the entire inner circumference of the pipe for complete coverage. The 90° jets provided larger force against the pipe surface (primary cleaning), while the 45° jets provided a flushing action to push the debris ahead of the nozzle. The cleaning head was mounted on the end of a 9.5-mm (3/8 inch) diameter hose that was attached to a pump. This pump could deliver up to 150 mL/s (2.3 gal per minute) at 14 MPa (2,000 psi) and could operate at temperatures of up to 88C (190F). The hose was fed through a stainless steel end cap that contained the hot water being flushed from the tube during cleaning. All components are approved for use with food products.

Giblets Collection

Giblets were obtained from a commercial processing plant and consisted of livers, hearts and pieces of gizzards. The giblets were transported

refrigerated to the Auburn University and were used in the experiments within 24 h of collection.

Sample Testing

To determine the natural contamination of the product, giblets samples were analyzed for *E. coli*/coliform (3M Microbiology Products, St. Paul, MN), total aerobes using APC (3M Microbiology Products) and *Campylobacter* spp. We determined the APC numbers using Petri film at 37C for 48 h, as suggested by the manufacturers (AOAC Official Methods 990.12, Aerobic Plate Count in Foods, Dry Rehydratable Film Methods), and *Campylobacter* enumeration was done using modified Campy-Cefex (mCC) agar plates. In all cases, 10 g of the product was mixed with 90 mL of sterile phosphate buffer solution (PBS) and stomached for 1 min. Serial 10-fold dilutions were made in PBS tubes and duplicate films or plates were inoculated from each dilution. *Escherichia coli*/Coliform and APC were incubated at 37C for 48 h. mCC plates were incubated at 42C with a gas mixture containing 10% CO₂, 5% O₂ and 85% N₂ (Airgas, Radnor, PA) in anaerobic jars gassed with a MACSmics Jar Gassing System (Microbiology International, Frederick, MD) for 48 h. A wet-mount was made with *Campylobacter*-presumptive colonies and the shape and motility of the bacteria under phase contrast microscopy were used to identify *Campylobacter* spp.

Sample Inoculation

Each trial was performed with 45 kg (100 lbs) of giblets, which was inoculated with 20 mL of PBS containing approximately 10⁷⁻⁸ cfu of *C. jejuni*. The 100 lbs were weighed and added to the hopper of the system over 1 min. When all giblets were added, the 20 mL of PBS with *Campylobacter* was added over 1 min. After thorough mixing in the pump and piping system (2 min), 10 g of the inoculated product was mixed with 90 mL of PBS, stomached for 1 min, serially diluted and spread plated in duplicate on mCC to count the number of cfu of *C. jejuni* per gram of product.

Experiments

Four independent experiments (performed on different days) were done where the 45 kg (100 lb) of inoculated product was circulated into the miniaturized transport system for 15 min. Figure 2 shows the product flow and the sampling scheme during the cleaning procedure. The system was then emptied and microbiology samples were taken from three areas of hopper pipe and three areas of the pump pipe. The samples were collected by swabbing two areas of 10 cm². The system was then cleaned with water at 60C (140F) that

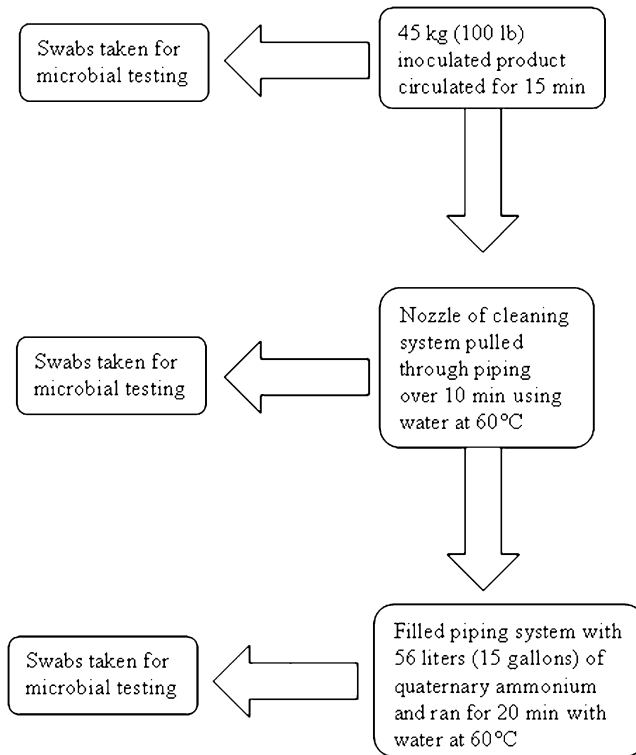


FIG. 2. EXPERIMENT, CLEANING PROCEDURES AND THE COLLECTION OF SAMPLES FOR MICROBIAL ANALYSIS

was run for 10 min. With the nozzle just inside the end of the pipe near the hopper, the hot water was turned on and the nozzle was pulled through the tube (approximately 2 min). After cleaning, samples for microbiological analysis were collected from the same two locations as those collected before cleaning. Briefly, an area of 10 cm² of the tubing adjacent to the pump and another 10 cm² area where the tubing enters the hopper were swabbed with sterile cotton swabs. The swabbed area was demarked by a 10-cm² frame and the swabbing was carried out for 10 s with a repetition of the swabbing at a 90° angle for 10 s.

Replications were done in different days. After each run, the equipment was cleaned up as described under Experiments section. The pipe was then cleaned with a conventional sanitizing process, which consisted of filling the piping system with 15 gal of a quaternary ammonium cleaning solution at a concentration of 195 ppm (R-Square Products, Inc, Gainesville, GA) at 60C

(140F) and pumping the solution through the piping system for 20 min. Three hot water 60C (140C) rinses were carried out to flush the tubing and pump. A third set of samples for microbiological analysis were collected after this cleaning process.

Microbial Testing

Swabs were immersed in 5 mL of PBS and the solution was analyzed for *E. coli*, coliforms, APC and *Campylobacter* by making serial dilutions and by following the protocols described under Sample Testing section.

Statistical Analysis

All bacterial counts were converted to \log_{10} cfu/mL. Analysis was done using analysis of variance procedure (SAS Institute Incorporated, Cary, NC), with significance set at $P \leq 0.05$. Means were analyzed for differences by Duncan's test (SAS Program).

RESULTS

The contamination of the giblets with naturally occurring bacteria is shown in Table 1. APC resulted in the highest bacterial numbers. The inoculation of *C. jejuni* resulted in an average of 10^5 cfu per gram of giblets, with a standard error of the mean of 0.69. The high level of inoculation was necessary to ensure that any reduction in *C. jejuni* could be quantified.

In the hopper pipe, statistical reductions in the number of bacteria were found for *C. jejuni* and APC only. The application of water resulted in a reduction of 2.2 \log_{10} cfu of *C. jejuni*. However, the quat. application yielded a reduction of only 0.6 \log_{10} cfu of *C. jejuni*. Although there was a reduction

TABLE 1.
AVERAGE MICROBIOLOGY ANALYSIS OF FOUR BATCHES
OF 45 KG (100 LBS) OF GIBLETS OBTAINED FROM A
COMMERCIAL PROCESSING PLANT

Organism	Log cfu/g of giblets (SEM)
Aerobic plate count	6.7 (0.81)
<i>Escherichia coli</i>	4.3 (1.49)
Coliforms	3.3 (1.33)

SEM, standard error of the mean.

in the number of *E. coli* and coliforms after the application of water and the quat, these reductions were not significant (Table 2).

Similar results were found for the pump pipe, although the only statistical reduction found was for *C. jejuni*. The combined cleaning of water plus a quaternary ammonium compound resulted in a reduction of 2 logs cfu of *C. jejuni* and APC, and more than 1 log cfu of *E. coli* and coliforms in the transport system.

DISCUSSION

The cleaning procedures are intended to reduce or eliminate pathogens and spoilage bacteria. We assessed the cleaning efficacy by enumerating APC, *E. coli*/coliforms and *C. jejuni* before and after the cleaning phases. The values of APC found in the product appear to be high in comparison with the other bacterial groups (Jay 2002); yet, there is a limited knowledge of the average APC for giblets products due to the lack of regular microbial testing of this product. Our results fall in the range of APC and *E. coli* counts found by Rodrigo *et al.* (2006) from naturally contaminated chicken livers from Trinidad.

The values obtained would be considered a concern from the food quality stand point. There is no uniformly recognized standard for performing APC or psychrotrophic plate counts (Jay 2002). The International Commission on Microbial Specifications for Foods recommends that APCs be incubated at 29–31C for 48 h. However, since 1985, many different temperature–time combinations have been utilized by researchers. The range of temperature–time incubations for APCs varies from 20C for 120 h to 37C for 24 h. It has also been demonstrated that isolates grown at one temperature do not grow at other temperatures and therefore are different bacterial populations (Jay 2002).

Campylobacter jejuni counts (inoculated) were approximately twice the natural count of chicken livers previously reported. Rodrigo *et al.* (2006) found 2.1 cfu/mL of *Campylobacter* in naturally contaminated chicken livers and hearts in Trinidad. Another study using the most probably number (MPN) technique showed that *Campylobacter* is found in numbers ranging from <3 MPN per 100 g to 10^2 – 10^3 MPN per 100 g in chicken livers (Whyte *et al.* 2006). Therefore, a higher inoculation was needed to quantify the possible reductions by the cleaning system.

The reduction found for the number of *C. jejuni* after cleaning suggests that this method may have a positive impact in the cleaning of actual giblets transport systems in poultry processing facilities. Yet, the reduction of APC and *E. coli*/coliform was not significant, nor consistent, to warrant a similar positive effect in an actual transport system.

TABLE 2.
EFFECT OF THE CLEANING DEVICE ON MEAN BACTERIAL COUNTS FOR FOUR INDEPENDENT EXPERIMENTS AND TWO PARTS OF THE GIBLETS TRANSPORT SYSTEM

System part*	Organism	Log ₁₀ cfu/g of giblets (SEM)			Reduction log ₁₀ cfu/mL		
		Precleaning	Post-cleaning water	Post cleaning quat†	Water	Quat	Total
Hopper pipe	<i>Campylobacter jejuni</i>	5.5 (0.85) ^A	3.3 (0.78) ^B	2.7 (0.59) ^B	2.2	0.6	2.8
	APC	6.3 (0.28) ^A	5.0 (0.13) ^B	4.6 (0.65) ^B	1.5	0.4	1.9
	<i>Escherichia coli</i>	3.5 (1.44) ^A	2.6 (0.97) ^A	2.2 (0.80) ^A	0.9	0.4	1.3
Pump pipe	Coliforms	4.1 (1.13) ^A	3.1 (1.24) ^A	3.1 (0.96) ^A	1.0	0.0	1.0
	<i>C. jejuni</i>	5.0 (0.40) ^A	3.0 (0.71) ^B	2.8 (0.62) ^B	2.0	0.2	2.2
	APC	6.5 (0.27) ^A	5.0 (1.16) ^A	4.5 (0.15) ^A	1.5	0.5	2.0
	<i>E. coli</i>	3.3 (1.35) ^A	2.8 (1.05) ^A	1.5 (0.50) ^A	0.5	1.3	1.8
	Coliforms	4.2 (1.21) ^A	2.9 (1.10) ^A	2.5 (1.26) ^A	1.3	0.4	1.7

Means in a row followed by different letters are statistically different ($P < 0.05$, Duncan's test).

* In each experiment, the giblets transport system was filled with 45 kg (100 lbs) of product and run for 15 min.

† Quat product, RQS Sanitizer (Quaternary Ammonium Product; R-Square) 37 mL (1.25 oz per 19 L (5 gal) of water at an average of 60C (140F). The giblets system was filled with approximately 57 L (15 gal) that were run through the system for 20 min.

APC, aerobic plate counts; SEM, standard error of the mean.

Although more research is needed to determine if chicken giblets or liver consumption is a significant vehicle for human campylobacteriosis, one study of New Zealand sheep livers and human campylobacteriosis cases showed that 61.1% of *C. jejuni* isolates from sheep livers were subtypes that were also isolated from human cases.

If giblets infected with *Campylobacter* make their way to human consumers, the most logical way to neutralize the threat of campylobacteriosis is through thorough cooking of giblets. It has been previously indicated that chicken livers held at a core temperature of 70C for 2–3 min are free of *Campylobacter*. This temperature and time combination leaves the center of the liver gray and perhaps unappealing to consumers, but the more appealing pink center may still harbor *Campylobacter* (Whyte *et al.* 2006).

In summary, the cleaning system designed in this study using only water at 60C (140F) that was run for 10 min. was able to significantly reduce both *C. jejuni* and APC in the piping system after simulated transport of chicken giblets. *Escherichia coli* and coliform counts show reductions, but these reductions were not statistically significant.

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