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Full Length Research paper

Existence of *Listeria* species in broiler carcasses with an attempt to control *Listeria monocytogenes* using trisodium phosphate

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The existence and control of *Listeria species* and *Listeria monocytogenes* on broiler's ceca, meat and skin at the retail level of Ismailia city, Egypt were studied. Dipping chicken filets in solution of (wt/vol) 0, 5, 10 or 15% Trisodium Phosphate (TSP) for 0, 3, 7 or 10 min were applied to chicken samples inoculated with *L. monocytogenes*. The results of this study revealed that 92, 42 and 70% of broiler's ceca, meat and skin were contaminated with *L. spp.* respectively. From the *L. spp.* positive samples, *L. monocytogenes* were the highest from ceca (60%, 30 of 50 samples), followed by skin (34%, 17 of 50), and meat (16%, 8 of 50). Dipping chicken filets into 0, 5, 10 or 15% TSP solution significantly reduced (P < 0.05) the counts of *L. monocytogenes* in treated samples as compared to the control. Dipping in 10 or 15% TSP for 10 min were significantly reduced (P < 0.05) *L. monocytogenes* counts to <100 cfu/g. The obtained results concluded that dipping in 10% TSP for 10 min appears to be an effective treatment for reducing populations of *L. monocytogenes* on broiler filets to a standard safe limit.

Key words: *L. monocytogenes*, trisodium phosphate, broiler, meat, filets.

INTRODUCTION

Microbiological safety and quality of broiler meat are equally important to producers, retailers and consumers. *Listeria monocytogenes* was the most common bacterial contaminant on broiler chickens at slaughterhouses (Lindblad et al., 2006). *L. monocytogenes* is particularly significant for cold-stored, ready-to-eat foods as it is frequently found in the environment and can grow at refrigerated temperatures. In spite of cleaning and disinfection after the chickens had been taken to the poultry slaughterhouse, microbial contamination from the intestinal contents occurred in the broiler houses in 16.9% of the cases (Dijkstra, 1978). Isolation of *L. monocytogenes* from chicken meat was recorded previously by many investigators (Keeratipibul and Lekroengsin, 2009; El-Shabacy Rasha, 2008; Lekroengsin et al., 2007; Akpolat et al., 2004 and Miettinen et al., 2001).

L. monocytogenes is an opportunistic pathogen that most often affects those with severe underlying conditions, pregnant women, unborn or newly delivered infants, and the elderly. Food-borne listeriosis is a relatively rare but serious disease with high fatality rates (20 - 30%) compared with other food borne microbial pathogens (WHO/FAO, 2004).

Preventive measures to avoid contamination of poultry products by *L. monocytogenes* must be taken in the processing plants (Rorvik et al., 2003). Inhibition of bacteria from carcass surfaces by frequent rinses with water and subsequent application of antimicrobials may be the most practical and effective means of improving the microbiological quality and safety of poultry meat. Trisodium Phosphate (TSP) is generally recognized as safe by the US Food and Drug Administration and has been approved by the US Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) at levels of 8 - 12% as an antimicrobial agent on raw chilled poultry carcasses that have been passed for wholesomeness (Federal Register, 1994). TSP (Na_3PO_4) has been evaluated by several investigators for its efficacy against pathogens attached to chicken carcasses and has been used as an effective compound in controlling the growth of Gram negative pathogens (Bin-Jasass, 2008).

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The results of Lindblad et al. (2006) improved the ability of researchers to assess the importance of chicken as a source of food borne pathogens and can serve as a basis for risk management actions. In addition, controlling *L. monocytogenes* on poultry meat has been identified as a specific targeted research need that will assist the industry in solving unique technical challenges within meat and poultry facilities (AMI, 2009). Therefore, this study was conducted to investigate the prevalence of *L. monocytogenes* and other *L. spp.* in ceca, meat and skin of broilers collected from Ismailia city, Egypt. A further goal was to evaluate the effectiveness of TSP treatment in reduction of *L. monocytogenes* load on chicken meat.

MATERIALS AND METHODS

Sampling

A total of 50 broilers were purchased and slaughtered upon request from six stores at Ismailia city. Slaughter was done using soft scald and hand picking evisceration. After that, broiler carcasses (without neck or leg) and their distal portion of ceca were placed separately in polyethylene bag and stored in an insulated box with gel-ice to keep the samples at 4°C or below while transported to the laboratory. In the laboratory a code number was given to each sample indicating the date of production. Additional samples of broiler filets (without skin) were collected from a poultry store within 20 min after slaughter. The filets were transported in a cooler to the laboratory. The filets were trimmed into pieces approximately 10 × 2.5 cm^2. Following excision, the samples were packaged in sterile oxygen impermeable 3 × 5 Whirl-Pak bags, and then frozen. Gamma irradiation with 12 kGy of ionizing radiation use a Cobalt-60 source was used to eliminate any background organisms on the filets. Once returned the samples were immediately frozen to -20°C and maintained at that temperature until using (Conner and Biligli 1994).

Microbiological analysis

The technique recommended by USDA-FSIS (1989) was adopted for isolation and identification of *L. monocytogenes*. Each 25 g of meat (random from broiler parts) or skin samples were mixed with 225 ml of sterile University Vermont Broth *(UVM1, Liofilchem*) and homogenized in a Seward Stomacher (400^R/UK) for 2 min to a 1:9 dilution (Wt/Vol). Similarly, 5 g of ceca sample were homogenized with 45 ml of UVM1 broth, and then all homogenized samples were incubated at 30°C for 24 h. Next, 0.1 mL of former incubated broth was transferred to 10 ml of University Vermont Broth (*UVM2, Liofilchem*) and re-incubated at 30°C for 24 h. A loopful from UMV2 broth was streak onto Supplemented Listeria PALCAM agar (*Liofilchem*) and incubated at 30°C for 24 h. Colonies showing bluish grey or black with a black halo and sunken center were picked up and streaked into Trypticase soy agar supplemented with yeast extract (Biolife) then incubated at 30°C for 24 h for purification (repeated twice) and identification.

Preparation of bacterial inoculums

One strain of *L. monocytogenes* previously isolated from the broiler carcass in this study was used. The strain was maintained on 10 ml Trypticase Soy agar supplemented with yeast extract (Biolife). Inoculated broths were incubated at 30°C for 24 h to achieve viable cell populations of 10^9 cells/ml. Following incubation, *L. monocytogenes* was prepared by diluting 10 ml of the suspension with 90 ml of sterile 0.1% (*w/v*) peptone water (Oxoid Ltd) to yield a final inoculum approximately 10^8 cells/ml.

Trisodium phosphate preparation

The appropriate amount of chemical for 5, 10 and 15% TSP (Food grade, Rhone-Poulenc, Cranbury, NJ), with sterile distilled water in a 150 ml beaker then the contents were placed into a 250 ml volumetric flask. The interior of the beaker was then rinsed three times with additional sterile distilled water and that rinse was also added to the flask. The flask was then filled to 250 ml mark with the appropriate volume of distilled sterile water. TSP solution were filter sterilized with 0.2 micron filters (Difco, Detriot, MI) and sealed into separate 500 ml pyrex screw capped sterile containers.

L. monocytogenes inoculation and TSP dipping

Samples were thawed and drip inoculated at different areas using a 1cc syringe with approximately 0.1 ml of an equal mixture of strains of *L. monocytogens*. Filets were placed and remained undisturbed on sterile plates at approximately 21°C for 10 min to allow for bacterial attachment. The inoculated samples were divided into four groups, each group containing 30 samples. Each group was dipped into TSP solution at concentrations of 0 (control), 5, 10 or 15% at 4°C for 0, 3, 7 or 10 min. Then, treated samples were drained by placed on a sterile refinery for 15 min at 4°C then microbio-logical analysis was done as former for counting the reduction rate (cfu/g).

Data analysis

The effects of treating with TSP (three concentrations, dipping time 0, 3, 5, 7 or 10 min), and their interactions on reduction of *L. monocytogenes* counts were analyzed by 2-way ANOVA of a completely randomized factorial design. The significance is determined at $P < 0.05$ (Sall and Lehman 2001).

RESULTS AND DISCUSSION

L. monocytogenes in broiler samples

Broiler carcasses can be contaminated with a variety of microorganisms from different sources during plant processes. In this study, *L. spp.* was commonly detected from broiler's ceca, meat and skin (Figure 1). The results revealed that 92%, 46 of 50 ceca, 42%, 21 of 50 meat and 70%, 35 of 50 skin samples were contaminated with *L. spp*. Many authors have demonstrated the widespread occurrence of *L. spp*. in raw broilers (Lindblad et al. 2006; Reiter et al. 2005; Antunes et al., 2002).

In this study, the higher detection rates for *L. spp*. on broiler samples may interoperated as their higher antibiotic resistance characteristic that enable the microbes to grow under the intensive antibiotics does given to broilers during rearing period (Lyon et al., 2008). In addition, the technological limitations during processing that can lead to cross-contaminations of the broilers carcasses being processed. The data obtained by Arritt (2000) suggested that due to current poultry plant procedures cross-contamination is virtually impossible to

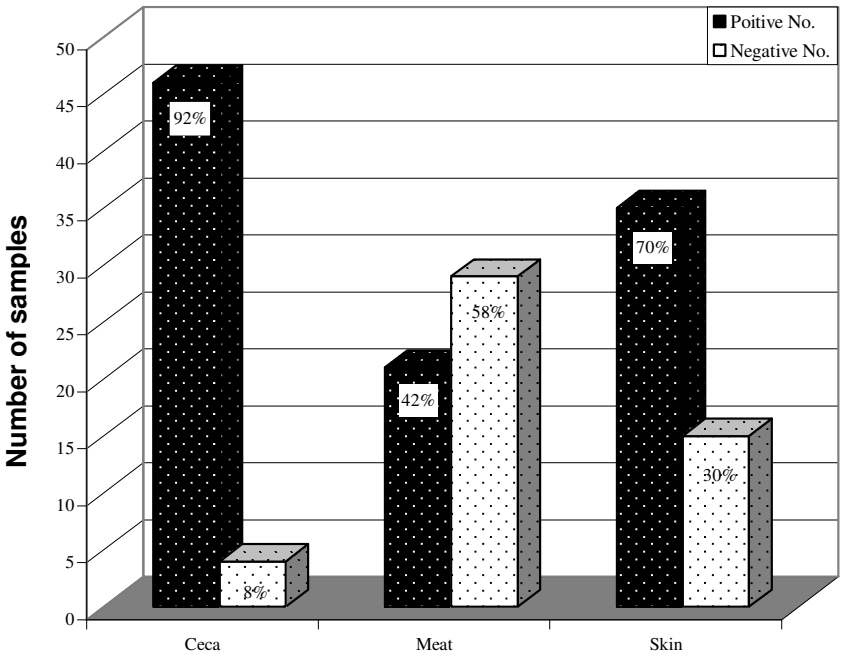

Figure 1. Percentage of *Listeria* spp. in broiler samples (n = 50).

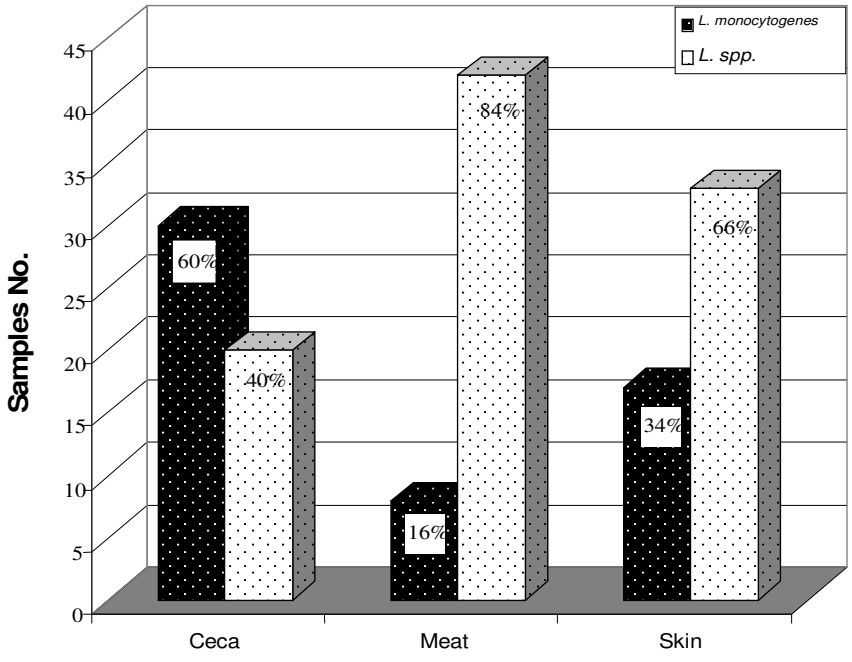

Figure 2. Percentages of *L. spp.* and *L. monocytogenes* among examined samples.

eliminate.

The results shown in Figure 2 revealed that 40, 84 and 66% of the 50 ceca, meat and skin samples analyzed tested positive for *L. spp.* respectively. For *L. monocytogenes*, all positive samples were from ceca (60%, 30 of 50 samples), skin (34%, 17 of 50), and meat (16%, 8 of 50).

L. monocytogenes have been isolated from many natural environments, such as water, soil, sewage, mud, birds and feces (Donnelly et al., 1992 and Nickelson and

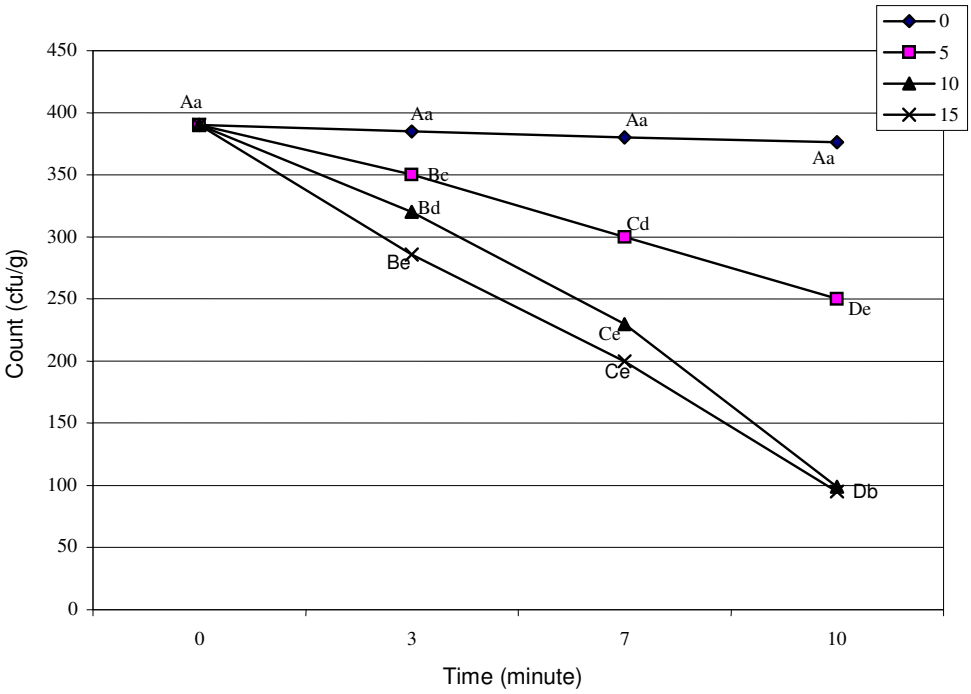

Figure 3. Efficacy of different concentration of TSP in reduction rate of *L. monocytogenes* inoculated into broiler fillets. Superscript capital letter compare between treatment periods in the same dose ($P < 0.05$) Superscript small litter compare between treatment doses in the same period ($P < 0.05$).

Finne, 1992), and it is considered an environmental contaminant. Moreover, Berrang et al. (2005) confirmed that raw product may be one source of *L. monocytogenes* in the poultry processing plant environment. Increased attention has been paid to *L. monocytogenes* since it was recognized as the food borne pathogen being responsible for human listeriosis (Jemmi and Keusch, 1994). Listeriosis can cause meningitis or septicemia in elderly or immuno-compromised persons, abortion, or illness in the newborn and mild gastrointestinal symptoms (FAO, 1999).

The frequency distribution of *L.* spp. among ceca, meat and skin were summarized in Table 1. The obtained results shown that 80, 24 and 42 *L. spp.* strains were isolated from ceca, meat and skin samples respectively. The highest frequency distribution for *L. monocytogenes* were seen in ceca (36, 45%) followed by skin (18, 42.9%) then meat (9, 37.5%).

Other *L. spp.* was isolated by different levels from examined samples. Lower incidence was recorded at Mengesha et al. (2009) who reported that of the 711 food samples examined, 189 (26.6%) were *Listeria* positive of which 34 (18%) were *L. monocytogenes*, sixteen percent of chicken carcasses were contaminated with *L. species*. Esteban et al. (2008) recorded 26.5% samples were positive for *L. monocytogenes* during their survey on 60 flocks of free-range chicken from 34 farms in the Basque Country (Northern Spain). The hazard of *L. monocytogenes* as human opportunistic pathogens has been confirmed (WHO/FAO, 2004), while the significance of other *L. spp.* towards broiler meat or consumers has not been recorded and still needs further investigation.

Efficacy of TSP on *L. monocytogenes*

The concentrations of the TSP and dipping time were a significant factor in reducing the populations of *L. monocytogenes*. Dipping broilers filets into different solution concentrations of TSP significantly reduced counts of *L. monocytogenes* as compared to the control (Figure 3). TSP has been approved by the United States Department of Agriculture as a pre-chill and post-chill antimicrobial treatment for raw poultry (Somers et al., 1994). TSP is a safe and effective chemical compound for bacterial reduction on meat, poultry and their products (Bin-Jasass 2008; Del Río et al., 2007; Del Río et al., 2006), and has significant bacterial reductions (marked in Gram-negative species) observed on TSP-treated samples throughout refrigerated storage (Del Río et al., 2005).

At three min dipping, there was no observed significant difference between 5, 10 and 15% concentration of TSP in reduction of *L. monocytogenes* counts. The effect of

Table 1. Frequency distribution of *L. monocytogenes* and other *L.* spp. among examined samples.

	Ceca		Meat		Skin	
	F	**%**	**F**	**%**	**F**	**%**
L. innocua	10	12.5	5	20.8	7	16.7
L. ivanovii	23	28.8	2	8.3	6	14.3
L. monocytogenes	36	45.0	9	37.5	18	42.9
L. murrayi	5	6.2	1	4.2	2	4.7
L. welshmeri	6	7.5	7	29.2	9	21.4
Total	**80**	**100**	**24**	**100**	**42**	**100**

TSP may be due to its high pH (pH 10), which affects the cell wall and the adherence of bacteria. TSP may also repress enzyme synthesis and inhibit enzyme activity of bacteria. After 7 min of dipping, results show significant differences between the treated groups and control. A significant reduction of *L. monocytogenes* counts was observed for solutions of 10 or 15% TSP as compared with 5% concentrations after 10 min of dipping. Hence, the best outcomes were attained with the highest TSP concentration studied (10 and 15%). At these concentration (10 and 15%), counts of *L. monocytogenes* were significantly reduced to < 100 cfu/g after 10 min of dipping. Egyptian Organization for Standardization and Quality Control (EOS, 2005) has not set an acceptable limit for counts of *L. monocytogenes* on poultry meat. However, European countries set acceptable limits below 100 cells per gram for products intended to be consumed after cooking (Pecciol et al., 2003). TSP solutions (10%) for 10 min. dipping reduced *L. monocytogenes* populations below the acceptable limits set by European countries. Similar results for inhibiting the growth of *L. monocytogenes* on chicken meat were obtained by Sallam and Samejima (2004) and Capita et al. (2003).

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