Fate of Small Populations of *Listeria monocytogenes* on Poultry Processed Using Moist Heat

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**ABSTRACT**

The survival of small populations of *Listeria monocytogenes* on poultry processed using a moist heating method was determined. Various inoculum concentrations (3.2 x 10², 4.8 x 10⁴, and 4.7 x 10⁴) were applied to chicken breasts which were cooked to an internal endpoint temperature of 73.9°C (165°F). After cooking, portions were either vacuum-packaged or wrapped in an oxygen permeable film and stored for up to 4 wk at 4°C or up to 10 d at 10°C. Some *Listeria* survived the cooking process regardless of the inoculum levels. Significant increases (p<0.05) in the *L. monocytogenes* population occurred for each inoculum concentration at both storage temperatures within the first sampling period (week 1 for 4°C and day 3 for 10°C). Samples stored at both temperatures were able to re-establish themselves to population levels at or above the initial inoculum. No differences were noted due to packaging.

Public awareness of food safety has increased due to documented outbreaks of listeriosis (3,7,20,21). Individuals whose health may be severely affected by listeriosis include pregnant women, newborns or young children, elderly persons or immunocompromised individuals. Healthy adults are usually little affected, if at all, by the presence of the bacterium, whereas the mortality rate in susceptible groups is approximately 30% (14).

*L. monocytogenes* possesses characteristics which enable its survival and proliferation at refrigerated temperatures. Therefore, refrigeration alone is not an effective preventative measure. The ubiquitous nature of *Listeria* is evident in the diverse habitats in which it can be found. Isolates have been found in various food products such as milk, red meats, poultry, seafood, vegetables, and fruits (17). The microorganism has been isolated from more than 35 mammalian species, as well as at least 18 avian species including domesticated chickens (6,11,23). *L. monocytogenes* has been isolated from sewage (12), decaying plant matter (23), 1-5% of the general population (19,21), poor quality silage (4,5,10,11), and effluents of abattoirs and poultry processing plants (22). Poultry is a potential vehicle for the transmission of listeriosis. Incidence rates of *L. monocytogenes* on poultry ranging from 15-60% have been reported (8,9,15).

Information on the behavior and survival characteristics of *Listeria* in processed food is needed in order to predict potential problems. The objective of this research was to study the survival of small populations of *L. monocytogenes* on chicken processed to 73.9°C (165°F) using a moist heating method. The fate of the microorganisms was determined for two packaging types (film overwrap vs. vacuum-packaging) and two storage temperatures (4 and 10°C).

**MATERIALS AND METHODS**

A stock culture of *L. monocytogenes* (Scott A) was maintained on trypticase soy agar slants at 4°C. The stock culture was transferred at 4-6 wk intervals. In preparation for inoculation onto fresh chicken breast, trypticase soy broth cultures were grown for 24 h at 37°C.

Fresh, raw boneless chicken breasts were purchased at an Athens, GA grocery and transported back to the laboratory on ice within 30 min. The inoculation and pre-heating treatment of the chicken breasts were performed as described previously (12), except three inoculation concentrations (3.2 x 10², 4.8 x 10⁴, 4.7 x 10⁴) of the Scott A strain were used. The various concentrations were prepared by serially diluting an overnight culture of *L. monocytogenes* to the desired levels.

The inoculated tissue was heated by placing the samples on a metal pan (24.5 x 41.0 cm) suspended in a second metal pan 1/3 full of water. The entire pan set was placed in a Reynold's Oven Cooking Rack (47.5 x 58.8 cm) and placed inside a pre-heated (255°C) Precision lab oven (Chicago, IL). Internal temperature of chicken breasts heated to 73.9°C (165°F) was monitored using thermocouples and a microprocessor. The cooking time was approximately 12-15 min. The packaging and storage of the cooked samples as well as the enumeration and confirmation of *L. monocytogenes* were performed as described previously (12). Lithium Chloride-Phenylethanol Moxalactam Agar (LPM, 16) was used as the selective medium for this microorganism. In addition, serial dilutions of the stomached samples were plated onto trypticase soy agar (TSA) using the spread plate technique to enumerate the total aerobic microflora. TSA plates were incubated at 37°C for 24 h.

Each cooking and packaging treatment was done three times. The data from the replicates were combined and analysis of variance (ANOVA) was used to determine if significant differences existed between microbial numbers during storage peri-
ods. When significant (p<0.05), mean counts were separated using Duncan’s multiple range test (18).

RESULTS AND DISCUSSION

The relationship between \textit{L. monocytogenes} populations obtained from LPM plates and background microflora obtained from TSA plates is illustrated for each inoculation level at each storage temperature (Fig. 1 and 2). Survivors were encountered at each inoculum concentration for both storage temperatures. For each inoculum concentration, net log reductions in \textit{L. monocytogenes} and total aerobic microflora populations due to the heat treatment were between 2 to 3.5. Within the first sampling period (week 1 for 4°C and day 3 for 10°C) there were significant increases (p<0.05) in \textit{L. monocytogenes} populations for each of the three inoculum levels regardless of storage temperature. The rate of increase for the \textit{L. monocytogenes} population over this time period was greater than the rate of increase for the total aerobic microflora for each treatment except for the samples initially inoculated with the smallest population and stored at 10°C (Fig. 2-A). The ability of \textit{L. monocytogenes} to survive thermal processing on poultry and proliferate during storage at 4 and 10°C has been demonstrated with initial \textit{Listeria} populations of approximately $1 \times 10^6$ to $1 \times 10^7$ (2,12).

The \textit{Listeria} population significantly increased to numbers above their respective initial concentrations in all samples except those that received an initial inoculum of $4.8 \times 10^3$ and were film overwrapped (Fig 1-B). The difference was greatest on those samples stored at the abusive temperature of 10°C, with populations at the end of the storage period 2.5 to 5.3 logs greater than the pre-heat population.

The increase in population differs from that reported by Johnson et al. (13) who found \textit{L. monocytogenes} did not proliferate and the population actually decreased in sausage stored at 4°C for up to 12 wk. The conflicting data is probably due to differences in substrate composition (e.g. presence of NaCl and a reduced pH in the sausage).

A concern with thermal processing is that pathogens may be sublethally injured. Therefore the number of \textit{L. monocytogenes} colonies counted on LPM agar at week/day 0 may not yield an accurate estimation of the total population of the organism present and may actually be an underestimation. Recovery of injured cells may occur within 4-6 h which could account for a portion of the viable \textit{L. monocytogenes} population in the samples by the first sampling period. These results add to the concern observed by Schlech et al. (20), that subjecting foods to prolonged cold storage could allow a small initial inoculum to multiply to a hazardous concentration.

Survival of small populations of \textit{L. monocytogenes} has been demonstrated. Other investigators have noted listerial populations in cook-chill foods at levels between 100 and 1000 organisms/g (14) and presence of between $10^4$-$10^5$ organisms/g in various cheeses (8). Initial populations of \textit{Listeria} used in this study are similar to those

![Figure 1. Growth of Listeria monocytogenes from cooked chicken packaged in a film overwrap (O) or vacuum-packaged (△) and the normal microflora packaged in a film overwrap (●) or vacuum-packaged (Δ). Samples were initially inoculated with $3.2 \times 10^3$ (A), $4.8 \times 10^3$ (B), or $4.7 \times 10^3$ (C) Listeria monocytogenes/g and were stored at 4°C after processing.](http://image.com/figure1.png)
naturally found in the environment. Low initial populations of *L. monocytogenes* on products processed to seemingly adequate temperatures can grow to concentrations surpassing initial numbers. This adaptation of the organism to its environment is of major concern since the infectious dose is presently unknown.

**REFERENCES**