RESEARCH REPORT

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PORK SAFETY

Title: Recovery of Listeria monocytogenes from packaged hot dogs
NPB# 99-228

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Stated Objectives:

1. Evaluate sampling methodologies for sensitivity in recovering *Listeria* spp.
2. Evaluate metabolic state of *Listeria* spp. after inoculation into packaged hot dogs, specifically in reference to the ability of current methodologies to recover the organism.
3. Refine existing procedures for detection of Listeria monocytogenes

Introduction:

A recent outbreak of listeriosis associated with hot dogs resulted in approximately 100 reported cases of the illness, with 21 of those cases ending in death (CDC, 1999). This outbreak along with subsequent recalls of other processed meat products for contamination with *Listeria monocytogenes* has resulted in a general concern with the bacterium. From a public health perspective, it is essential to address the issues of contamination of meat products with *Listeria* spp.

One of the essential elements in this process is the ability to reliably detect the presence of the bacterium on processed meats. It is known that the levels of Listeria monocytogenes in product from the same lot as the product implicated in the recent outbreak were very low, with reports of as few as 6 colony forming units per hot dog. Detection of very low numbers of bacteria requires a through evaluation of not only the statistical validity of the sampling plan, but also an evaluation of the microbiological sampling methodology to assure detection of the organism.

Anecdotal evidence from industry and academic laboratories suggests that there may be a phenomenon occurring with *Listeria* spp. which may adversely impact detection of the pathogen. Specifically, several laboratories have apparently observed that when *Listeria* spp. is experimentally inoculated in low numbers onto processed meats, it is not recoverable after a few days storage at refrigeration temperatures. However, after extended storage at refrigeration temperatures, *Listeria* spp. is recoverable from the experimentally inoculated packages.

These observations suggest two hypothesis: (a) very low numbers of *Listeria* spp. may not be recoverable by current sampling protocols or (b) the processing aids incorporated into processed meat formulations, in combination with vacuum packaging, may exert environmental stress on the bacteria and injure them to the point that standard recovery procedures kill the bacteria they are designed to recover. There is a previously documented condition in some environmental bacteria which has been identified as "viable, non-culturable" (Roszak et al., 1984; Wolf and Oliver, 1992), and there is at least a preliminary basis to suggest that this may be occurring with *Listeria* spp. in processed meats. Alternately, the *Listeria* may simply be injured by the environmental conditions, and therefore may find the restrictive growth conditions in the UVM primary enrichment media inhibitory. This would suggest the incorporation of a non-selective, pre-enrichment step in the methodology, similar to the pre-enrichment step used in the salmonellae procedure (USDA-FSIS, 1998).

References:

Centers for Disease Control and Prevention. 1999. Update: Mulitstate outbreak of Listeriosis. www.cdc.gov/od/oc/media/pressrel/r990114.htm

Roszak, D.B., D.J. Grimes and R. R. Colwell. 1984. Viable but nonrecoverable stage of Salmonella eteritidis in aquatic systems. Can. J. Microbiol. 30:334-338.

USDA-FSIS. 1998. Isolation and identification of *Salmonella* from meat and poultry. Microbiological Laboratory Guidebook, 3rd. edition.

Wolf, P. W. and J. D. Oliver. 1992. Temperature effects on the viable but non-culturable state of Vibrio vulnificus. FEMS Microbiology Ecology 101:33-39.

Materials and Methods

I. Sampling methodologies: Hot dogs sterilized by ionizing radiation were inoculated with a five strain mixture of *Listeria monocytogenes* at three different inoculum levels) and then vacuum packaged. The hot dogs were incubated at 3C for 24 and 72 hours, and then sampled by aseptic excision of all external surfaces, swabbing with a sterile sponge and rinsing with 20 ml sterile buffered peptone water. The data were converted to $\log_{10}$ colony forming units/cm^2 (the average surface area of a hot dog is 100 cm^2), and then analyzed by the general linear models procedure of SAS.

II. Characterization of Metabolic state: Hot dogs sterilized by ionizing radiation were inoculated with a five strain mixture of *Listeria monocytogenes*, and then vacuum packaged. The hot dogs were incubated at 3C for 24 and 72 hours, and evaluated against 0 hour control samples. The hot dogs were sampled by rinsing, based on the results of Objective I, and the populations enumerated on TSA-YE (non-selective), TSA-YE +5% NaCl (selective) and MOX (selective). The difference in populations between the non-selective and selective media was as an indication of injury.

III. Refinement of Recovery Methods: A non-selective pre-enrichment step was added to the recovery procedure, prior to enumeration. Radiation sterilized hot dogs were inoculated, vacuum packaged and held for 24 hours at 3C. The samples were rinsed as described above, and the rinse added to 20 mls of double strength buffered peptone

water (BPW) or Universal Pre-enrichment Broth (UPE). The broths were held at room temperature, and at 0, 2,4, and 6 hours, samples were enumerated on tryptic soy agar with yeast extract (TSAYE) an modified Oxford medium (MOX).

Results and Discussion

The results of the comparison of sampling methods are shown in Figure 1. Rinsing and full surface excision were statistically equivalent, while swabbing produced statistically lower populations. Based on the relative ease of use, rinsing was used for all further experiments.

The experiments conducted to evaluate metabolic state did not indicate the presence of injured *Listeria monocytogenes*. That is, the populations were equivalent on both non-selective and selective agar at all of the sampling times (data not shown). A likely explanation for these results is that the initial bacteria used for inoculation purposes were grown and maintained under optimal conditions. These "healthy" bacteria were more resistant to the environmental stresses encountered on the surface of a vacuum packaged hot dog than bacteria which may enter from the environment. Random environmental contaminants would most likely be in a stressed metabolic state initially, and would therefore be more sensitive to the stresses encountered on the hot dog surface.

Although metabolic injury could not be replicated in the laboratory, experiments were conducted to determine the practicality of adding a non-selective pre-enrichment step to the recovery procedures. The addition of a pre-enrichment step with either buffered peptone water or universal pre-enrichment broth were statistically equivalent and did not result in a numerical increase in bacterial populations for up to 4 hours on TSAYE (Fig. 2). The recovered populations on MOX were initially lower than the TSAYE, but increased over incubation time (Figure 3). There was no statistical difference between the recovery on either BPW or UPE on MOX agar. These results could be interpreted as enhancing recovery on selective media, as a result of repairing slight metabolic damage or providing necessary nutrients for the bacteria to grow on the medium. The results on the non-selective media suggest that the actual population in the pre-enrichment did not increase until 6 hours of incubation, so the increase in populations on the selective agar are an indication of recovery or enhanced growth under restrictive conditions. These results suggest that a non-selective pre-enrichment procedure, consisting of buffered peptone water or universal pre-enrichment broth, could be added to enhance recovery of *Listeria* when it is suspected that they could be metabolically injured. This pre-enrichment step would be conducted at room temperature for up to 4 hours.

Summary:

The results of this study indicate that rinsing hot dogs is an acceptable sampling technique to detect surface *Listeria*. This technique is relatively rapid and requires little laboratory equipment. Although the initial premise of the study, namely that Listeria which contaminate the surface of hot dogs are metabolically injured, there still is the distinct possibility that it does affect the outcome of sampling and testing procedures. The inclusion of a non-selective pre-enrichment step in the detection procedures for *Listeria* may be beneficial, when the presence of injured bacteria is suspected.

Figure 1. Comparison of Sampling Methods for the Recovery of *Listeria monocytogenes* from hot dog surfaces.

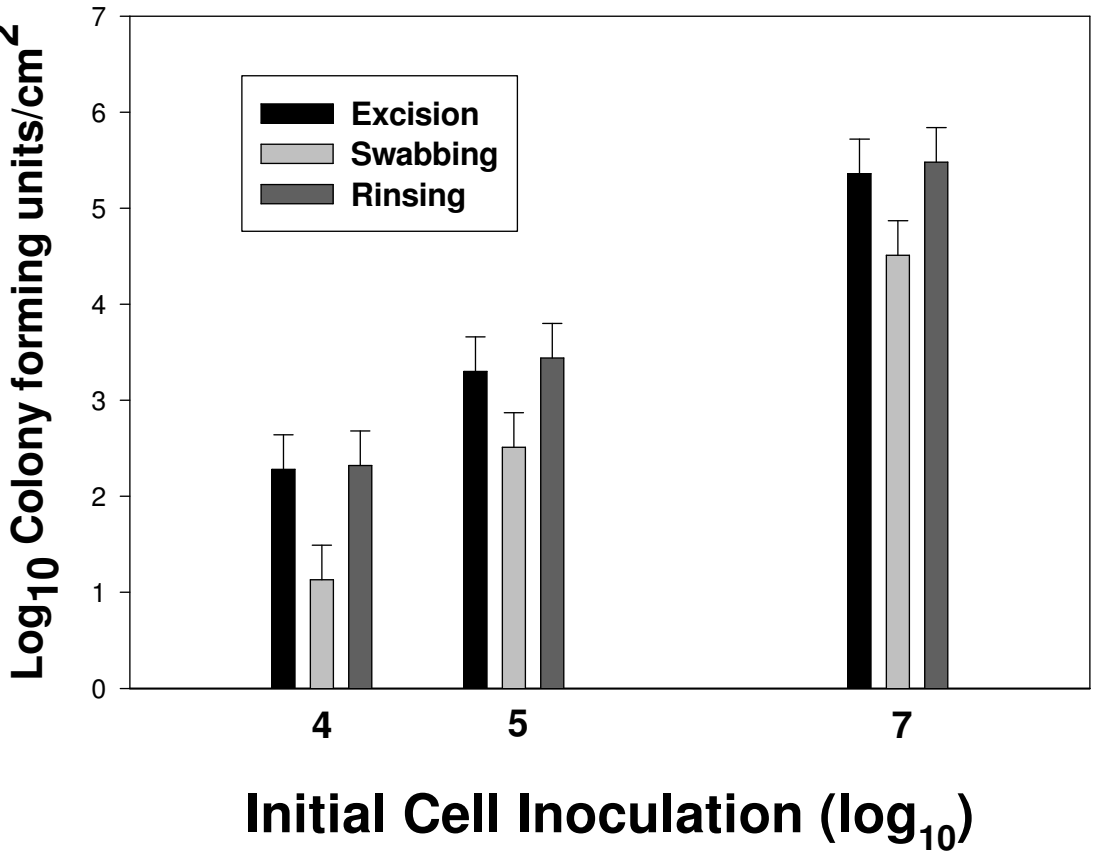

Figure 2. Populations of *Listeria monocytogenes* Recovered from hot dogs on TSAYE with the addition of buffered peptone water (BPW) or Universal Pre-enrichment broth (UPE).

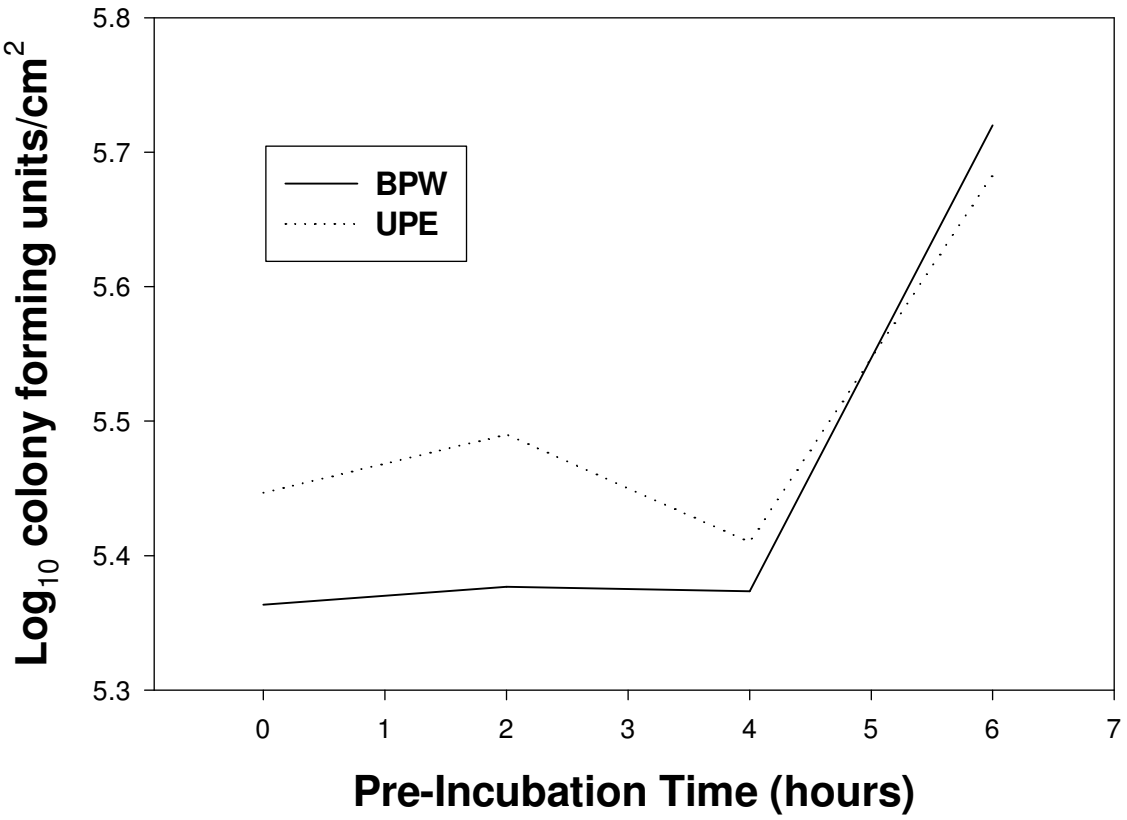

Figure 3. Populations of *Listeria monocytogenes* Recovered from hot dogs on MOX with the addition of buffered peptone water (BPW) or Universal Pre-enrichment broth (UPE).