Rapid Detection of *Listeria monocytogenes* in Food and Food Animals

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Summary and Implications

The objective of this study was to stream- line the detection and identification of *Listeria monocytogenes* (LM) in livestock and foods. Hog tonsil scrapings, hog tissues collected at necropsy, ground pork, and turkey washes were inoculated into UVM-1 (10% w/v) and after incubation (3 days) transferred to UVM-2. After 2 days, samples were plated to Palcam agar and incubated (48 h, 37°C). Characteristic LM colonies were verified with the multiplex Polymerase chain reaction assay, which targets the 16S rRNA gene of *Listeria* and the *hlyA* gene unique to the species *monocytogenes.* To determine when LM achieved detectable levels during enrichment, template DNA was extracted directly from UVM-2 and Palcam agar and screened by multiplex PCR. When screened directly from UVM-2 by PCR, 34% (65 of 190) of ground pork samples and 19% (11 of 60) of turkey washes were positive for LM. In contrast, by multiplex PCR screening of colonies from Palcam agar 39% (75 of 190) of ground pork and 29% (18 of 60) of turkey wash samples were positive. Future studies will focus on improved recovery from UVM-2 with immunomagnetic beads. LM positive isolates (n=33) of ground pork were serotyped and assigned to type 4 (72%) and type 1 (14%). Forteen percent of isolates were neither serotype 1 nor 4. For live hogs, out of 150 samples each of carcasses, tonsils, and ileocecal lymph nodes tested, LM was detected once from tonsils and nodes by multiplex PCR. In contrast, LM was found on ~30% of ground pork produced from that same day from packing plant.

Introduction

LM is a gram-positive, facultative intracellular bacterium that can cause severe infections in humans and several other animal species. LM has a special predilection for immunocompromised individuals. It is a food-borne pathogen that is ubiquitous and as such, is a food contaminant. Although infrequently present in hogs, LM colonizes the tonsils (5). LM was found on 7.4% of hog carcasses during the Food Safety and Inspection Services microbial baseline studies (4). Listeriosis outbreaks have increased the need for rapid, highly sensitive methods for the detection and identification of LM (2). The use of PCR as a diagnostic tool for the detection of pathogens has expanded in recent years. The purpose of this study was to develop and evaluate rapid methods to isolate and confirm LM in hog tissues, ground pork, and turkey washes.

Materials and Methods

Live hogs (n=280) from a commercial herd, ground pork (n=340), and turkey washes (n=80) from packing plants were analyzed.

Samples from hog tissues at necropsy. Market weight hogs (n=280) were screened. At the farm tonsil scrapings were collected from market weight hogs (n=30). Half (n=15) remained at the farm as controls with access to feed and water. The other half (n=15) were lairaged overnight at the National Animal Disease Center with access to water only (experimental). The next day all 30 hogs (control + experimental) were transported to the same packing plant and slaughtered. This was repeated for 10 weeks. At the abattoir, tonsils, ileocecal, superficial inguinal, ventrothoracic lymph nodes, rectal contents, and carcass swabs were collected and transported to laboratory. Lymph nodes were homogenized in 25 ml of peptone water (pH 7.4) and an aliquot (1 ml) placed into 9 ml of UVM-1. One gram of rectal contents and carcass swab samples (~ 20% of sponge used to swab carcass) enriched in 9 ml of UVM-1 (30°C, 3 days). Enrichments were subcultured to UVM-2.

Samples of ground pork. Seven trials were conducted for ground pork produced at the packing plant on the same day as slaughter. A total of 340 samples were analyzed. Each sample (25 g) was enriched in 225 ml of UVM-1 (30°C, 3 days) and subcultured to UVM-2.

Samples of turkey washes. Eighty turkey carcass washes were analyzed. The samples were collected by plant personnel and shipped overnight on ice to the laboratory. Once received 200 ml of sample was pelleted (16,000*g* for 15 min), resuspended into 10 ml of peptone water, and an aliquot (1 ml) placed in 100 ml of UVM-1 (30°C, 3 days). Enrichments were subcultured to UVM-2.

Listeria spp. enrichment technique. A two-step enrichment procedure was followed by subculturing on Palcam. After enrichment and incubation of UVM-1 (30°C for 3 days), 1 ml was transferred to UVM-2 and incubated (30°C for 2 days). After incubation, 1 ml from

UVM-2 was collected for PCR. A loopful was plated to Palcam and incubated microaerobically (10% CO_2, 5% O_2, 84% N_2) for 48 hrs at 37°C.

Preparation of bacterial DNA. Aliquots (1 ml) taken from UVM-2 enrichment were centrifuged (14,000*g* for 2 min), the supernate decanted, and the pellet resuspended in 250µl of sterile distilled water. UVM-2 enrichments were subsequently plated to Palcam agar. Suspect *Listeria* (colonies surrounded by black halos from aesculin hydrolysis) were swiped and suspended in 250µl of sterile distilled water. For PCR analysis, samples from both enrichments and plates were boiled in a water bath (10 - 12 min), centrifuged (14,000 *g* for 1 min), and the supernatant used as the PCR template.

PCR analysis. Two primer sets were used: set I (primers Lis-1 and Lis-2) specifically amplifies 174-bp region of the listeriolysin gene (*hlyA*) of LM (3). Set II (primers U1 and LI1) targets a 938-bp region encoding the 16s rRNA gene of *Listeria* (1). Amplification conditions were as follows: initial denaturation step at 96°C for 4 min, followed by 30 amplification cycles. Each cycle consisted of denaturation (94°C for 1 min), primer annealing (60°C for 45 s), and primer extension (72°C for 1 min). The amplified DNA was analyzed by gel electrophoresis (100 V for 55 min) on 2% agarose gels with Tris-borate EDTA (TBE 1×) as the running buffer. The gels were stained with ethidium bromide, rinsed and visualized on a Gel Doc 1000 (Bio-Rad Laboratories, Richmond, CA).

Results and Discussion

Table 1 summarizes the recovery of *Listeria* and LM from UVM-2 and Palcam. For hog tissues, only LM was detected in 1.3% of tissues (n=150) from both UVM-2 and Palcam. For ground pork collected that same day from the abattoir, UVM-2 enrichments harbored *Listeria* (2.6%) and LM (31.5%). The incidence of LM in ground pork is higher compared with tissues of hogs slaughtered on that same day. This may be due to post-slaughter contamination. LM positive isolates (n=33) of ground pork were serotyped and assigned to type 4 (72%) and type 1 (14%); 14% isolates were neither serotype 1 nor 4. UVM-2 enrichments were subsequently plated to Palcam agar. Thirty-nine percent of ground pork samples from Palcam were positive; 0.5% were *Listeria* spp and 38.5% were LM. Turkey washes tested to compare the recovery from UVM-2 and Palcam. Overall, 29% of turkey carcass rinses were positive; 21% were *Listeria* spp and 8% were LM. In general, recoveries were slightly higher from Palcam, although the differences were not statistically significant because of the small sample size.

Acknowledgements

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References

1. Border, P. M., J. J. Howard, G.S. Plastrow, and K.W. Siggens. 1990. Detection of *Listeria* species and *Listeria monocytogenes* using polymerase chain reaction. Lett. Appl. Microbiol. 11: 158 - 162.
2. Czajka, J. and C. A. Batt. 1994. Verification of casual relationships between *Listeria monocytogenes* isolates implicated in food borne outbreaks of listeriosis by randomly amplified polymorphic DNA patterns. J. Clin. Microbiol. 32: 1280 - 1287.
3. Deneer, H. G., and I. Boychuk. 1991. Species-specific detection of *Listeria monocytogenes* by DNA amplification. Appl. Environ. Microbiol. 57: 606 - 609.
4. USDA-FSIS microbial baseline studies. 1996.
5. Wesley, I.V 1998. Listeriosis in animals. *Listeria*, Listeriosis, and Food Safety. 2: 39 – 73.

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Figure 1. Summary of protocol for *Listeria* enrichment and growth.

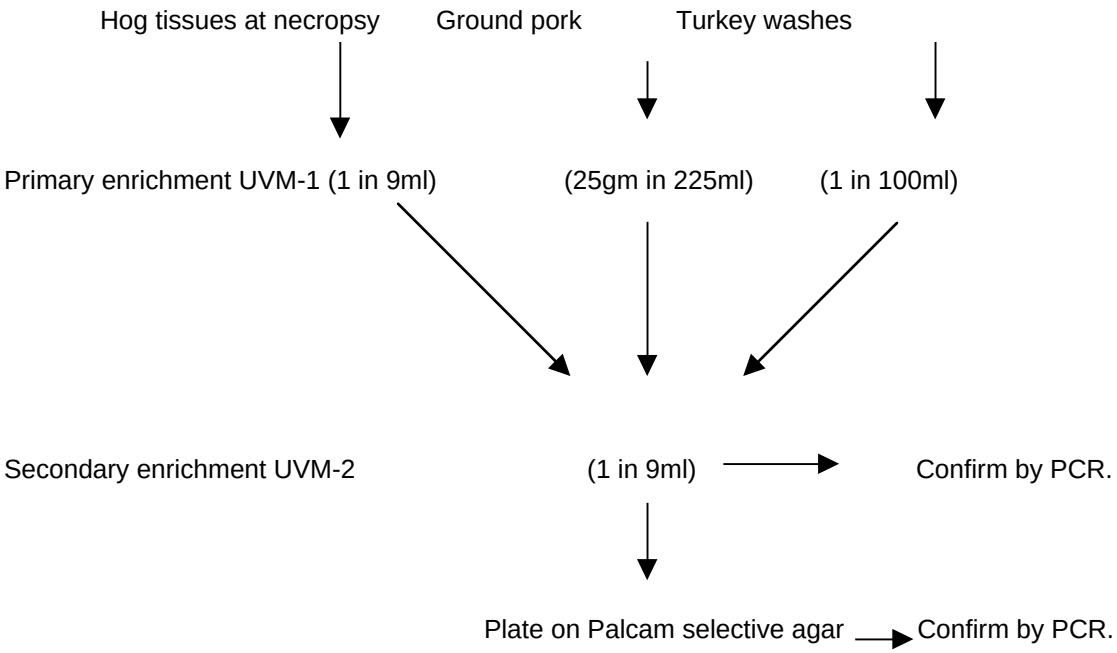

Table 1. Summary of detection of *Listeria* and *L. monocytogenes* in UVM 2 and plating to Palcam agar by multiplex PCR.

Sample number examined	**UVM 2**		**Palcam**	
	Listeria spp	*L. monocytogenes*	*Listeria spp*	*L. monocytogenes*
Hog tissues (n=280)	0 (0%)	16 (5.7%)	3 (1.07%)	46(16.4%)
Ground pork (n=340)	5 (1.5%)	151 (44.41%)	6 (1.7%)	171 (50.2%)
Turkey washes (n=80)	9 (11.25%)	7 (8.75%)	19 (23.7%)	12 (15%)